

Respiratory pathophysiologic responses

The contribution of neurogenic reflexes to hypertonic saline-induced bronchoconstriction in asthma

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Background: Hypertonic saline induces bronchoconstriction in most patients with asthma; however, the mechanisms involved are not clearly understood. We have investigated the role of neurogenic mechanisms in hypertonic saline-induced bronchoconstriction.

Methods: The effect of pretreatment with the anticholinergic drugs atropine and ipratropium bromide and the local anesthetic drug lidocaine on hypertonic saline responsiveness was studied in 11 subjects with asthma. Ultrasonically nebulized hypertonic saline challenge was given in a dose-response manner to determine the provocative dose of hypertonic saline-laden air required to produce a fall in forced expiratory volume in 1 second (FEV₁) of 20% or greater (PD₂₀HS). A control PD₂₀HS with no pretreatment was measured before randomization, and on the next three visits PD₂₀HS was determined after pretreatment with each of the following: intramuscularly administered atropine, inhaled ipratropium bromide, and inhaled lidocaine.

Results: The baseline mean FEV₁ values increased by 3.5% and 4% 30 minutes after administration of atropine and ipratropium bromide, respectively, and decreased by 5.8% at 10 minutes after inhalation of lidocaine. These changes in the baseline FEV₁ were not significant and did not have any effect on the PD₂₀HS response. Premedication with atropine, ipratropium bromide, and lidocaine resulted in increases of 2.5, 2.0, and 2.6 times in mean PD₂₀HS, respectively.

Conclusions: The protection afforded by the drugs was variable in the individual subjects and not related to age, sex, baseline FEV₁, provocative concentration of histamine causing a 20% fall in FEV₁ or use of the inhaled corticosteroids. The protection afforded by anticholinergic and local anesthetic drugs suggests the contribution of neurogenic reflexes in the pathogenesis of hypertonic saline-induced bronchoconstriction in asthma. (*J ALLERGY CLIN IMMUNOL* 1993;92:82-8.)

Key words: Asthma, hypertonic challenge, atropine, ipratropium bromide, lidocaine

Hypertonic saline aerosol challenge has been shown to be an effective bronchoconstrictor stimulus in asthma.^{1, 2} However, the mechanisms by which hypertonicity causes bronchoconstriction are not known. Suggested mechanisms include mediator release from primed mucosal mast cells and stimulation of neurogenic reflexes facilitated by the loss of epithelial integrity that occurs in

Abbreviations used:

- FEV₁: Forced expiratory volume in 1 second
- PD₂₀HS: Provocative dose of hypertonic saline-laden air required to produce 20% fall in baseline FEV₁
- EIA: Exercise-induced asthma

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asthma.³ The parasympathetic system is an important neural bronchoconstrictor mechanism and along with peptidergic pathways plays an important role in the regulation of airway tone.⁴ It has been shown that hypertonic saline causes neurogenic inflammation in the eye in human beings⁵ and in the rat trachea.⁶

TABLE I. Characteristics of subjects studied

Subject No.	Age (yr)	Sex	Atopy	FEV ₁		PC ₂₀ histamine (mg/ml)	Medication†
				(L)	(% predicted)		
1	22	F	DP,HD	3.90	109	1.58	S prn
2	43	M	DP,CF,GP	3.85	92	0.89	S prn
3	34	F	HD,DP,GP	3.40	108	3.40	S prn
4	24	F	HD	2.20	76	0.04	S, prn; B, 500 µg bd
5	24	M	HD,DP,GP	2.50	84	5.78	S prn
6	36	F	DP,GP,F	3.95	99	2.41	S, prn, B, 500 µg bd
7	23	F	HD,PD,GP	3.65	105	13.7	S prn
8	23	M	DP,HD,GP	3.20	89	0.34	S, prn; B, 100 µg bd
9	25	M	DP,CF,GP	6.10	125	0.92	S prn
10	19	M	DP,HD	3.72	77	0.68	S prn
11	24	M	HD,DP,GP	3.75	77	2.80	S prn
Mean ± SEM		27 ± 2		3.65 ± 0.30	94.6 ± 4.80	1.32* ± 0.20	

DP, *Dermatophagoides pteronyssinus*; HD, house dust; S, inhaled salbutamol; prn, as needed; CF, cat fur; GP, grass pollen; F, feather; B, inhaled beclomethasone; bd, twice daily.

*Geometric mean.

A few studies have reported the effect of anticholinergic agents on hypertonic saline-induced bronchoconstriction. Anderson et al.¹ reported no overall significant protection with inhaled atropine in a group of patients with asthma but did find large degrees of protection in four of the nine subjects studied. Inhaled ipratropium bromide has been shown to significantly reduce the bronchoconstrictor response to hypertonic saline,⁷ and Freed et al.⁸ have demonstrated that atropine attenuates the response to hypertonic saline-induced bronchoconstriction in the peripheral airways of dogs.

The effect of topical lidocaine on hypertonic saline-induced bronchoconstriction has not previously been studied. It has been suggested that sensory irritant or thermally sensitive receptors in the respiratory tract are involved in the pathogenesis of exercise-induced asthma (EIA).^{9, 10} There is no consensus on the protection afforded in EIA by blocking sensory irritant receptors in the airways with lidocaine,^{11, 12} and Sheppard et al.¹³ could not demonstrate that lidocaine significantly attenuated bronchoconstriction induced by hypotonic saline.

In the present study we have determined the contribution of neurogenic mechanisms in hypertonic saline-induced bronchoconstriction by observing the effect of the antimuscarinic drugs atropine and ipratropium bromide and the local anaesthetic agent lidocaine on the airway responsiveness to hypertonic saline.

METHODS

Subjects

Eleven subjects with asthma (six women and five men) with a mean (± SEM) age of 27 (± 2.2) years and a history of EIA participated in the study. All subjects were nonsmokers with atopic asthma as judged by at least one wheal greater than 3 mm in diameter on skin prick test with *Dermatophagoides pteronyssinus*, house dust, mixed grass pollen, dog, feather, and cat extracts (Bencard Allergy Unit, Brentford, Middlesex, England). Their baseline forced expiratory volume in 1 second (FEV₁) was greater than 75% of their predicted values, and none were receiving corticosteroids or theophylline orally (Table I). Treatment with inhaled β₂-agonists was withdrawn at least 8 hours before each visit to the laboratory, although subjects were allowed to continue administration of inhaled corticosteroids as usual. Patients were not studied within 4 weeks of an upper respiratory tract infection or exacerbation of their asthma. The study was approved by the Southampton Hospitals and University Joint Ethics Subcommittee, and written informed consent was given by all subjects involved in the study.

Study design

The study was divided into two phases.

Phase 1. The initial phase comprised two separate nonrandomized visits to the laboratory, at least 72 hours apart, to undertake dose-response studies with inhaled histamine and hypertonic saline in order to establish the baseline level of response. Only subjects whose provocative dose of hypertonic saline-laden air required to produce a 20% fall in FEV₁ (PD₂₀HS) was less than 310 L were included for phase 2 of the study.

Phase 2. The subjects attended the laboratory on 3 separate days at the same time of day for evaluation of the effect of drug treatment on hypertonic saline responsiveness. On one of each of the study days, subjects received in random order atropine, ipratropium bromide, or lidocaine. Before drug treatment three baseline measurements of FEV₁ were made at 3-minute intervals, and the highest value was recorded. Dose-response challenges with hypertonic saline were performed on each study day at least 3 days apart. The greatest of three FEV₁ estimations made immediately before administration of hypertonic saline was taken as baseline value.

Premedication. The dose, route, and time interval for administration of atropine, ipratropium bromide, and lidocaine were the same as those in use for the premedication for bronchoscopy challenge in our unit. Atropine (atropine sulfate injection [British Pharmacopial], Antigen Pharmaceuticals Ltd.) was given intramuscularly in a dose of 0.6 mg. Ipratropium bromide 500 µg in 2 ml of isotonic solution (Boehringer Ingelheim Ltd.) and lignocaine (2 ml lidocaine hydrochloride 4% wt/vol topical [Birex], pH = 5.22, osmolality = 274 mosmol/kg H₂O) were given as an aerosol by inhalation. Atropine and ipratropium exert their maximum bronchodilator effect at 30 minutes. The effect of these drugs on hypertonic saline challenge was studied at 30 minutes after the dose was given to determine whether bronchodilation caused by the drugs contributed to the protection. However, local anesthetic effect of lidocaine peaks between 2 and 5 minutes and lasts for 30 to 45 minutes; its protective effect was studied at 10 minutes.

The aerosols were delivered from an Inspiron nebulizer (C.R. Bard International, Ltd., Sunderland, England) driven by compressed air at flow of 8 L/min, producing a nebulizer output of 0.33 ml/min and generating an aerosol with a mass median particle diameter of 4.1 µm.¹⁴ The drug aerosols were inhaled from functional residual volume to total lung capacity, and inhalation continued until complete nebulization of the solution was achieved.

Bronchial provocation tests

Histamine bronchial challenge. Histamine acid monophosphate (BDH Chemicals, Poole, Dorset, England) was made up in 0.9% sodium chloride in a series of doubling concentrations, ranging from 0.03 to 16 mg/ml (0.1 to 52.1 mmol/L). The solution was administered as an aerosol generated from a starting volume of 3 ml in an Inspiron nebulizer. Bronchial challenge was performed with a modification of the technique of Chai et al.¹⁵ Before challenge, a baseline FEV₁ was obtained, which was the highest of three technically satisfactory records with a dry wedge spirometer (Vitalograph, Buckingham, England). Subjects then took five breaths of 0.9% sodium chloride (normal saline) from functional residual volume to total lung capacity, from the nebulizer. Two measurements of FEV₁ were

performed at 3 minutes, and the higher value was accepted. If the FEV₁ after saline challenge was within 10% of baseline, then the histamine challenge was undertaken. Increasing doubling concentrations of histamine from 0.03 to 16 mg/ml were administered at 5-minute intervals until a 20% fall in FEV₁ from the value after saline challenge was achieved. From the plot of percentage fall in FEV₁ against the natural logarithm of the cumulative agonist concentration, the provocative concentration of histamine causing a 20% fall in FEV₁ (PC₂₀ histamine) was derived by linear interpolation.

Hypertonic saline bronchial challenge. Hypertonic saline (3.6%) was aerosolized with a DeVilbiss model 65 ultrasonic nebulizer (DeVilbiss, Feltham, Middlesex, England), which has an output of 10.8 ml per 100 L of air at the chosen nebulizer setting. A previous study in our laboratory has shown that the output of hypertonic saline to the mouthpiece is linearly related to the volume of air drawn through the nebulizer.¹⁶

The input port of the nebulizer chamber was connected to a Parkinson Cowan gas meter (PK Morgan, Chatham, Kent, England) by wide-bore corrugated tubing with an internal diameter of 4 cm. The signal from the gas meter was input to a BBC microcomputer, which converted the reading to body temperature and pressure and saturated with water vapor and displayed the volume in real time on a monitor. The output port of the nebulizer was connected to a two-way valve so that expired air could be ducted out of the laboratory. Thus when the nebulizer was in operation, the subject inspired air that contained nebulized saline, and the cumulative volume of the inspired air was simultaneously displayed on the computer monitor.

The hypertonic saline challenge was given in a dose-response manner. Two measurements of FEV₁ were made for each subject, and the higher reading was used as the first baseline value. The subject was then asked to breathe at tidal volume through the mouthpiece, with the nebulizer switched off, until 25 L of air had been respired. Two additional measurements of FEV₁ were made, and the higher reading was taken as the new baseline value. If the new baseline value differed from the first by more than 10%, then the procedure was discontinued, although this was not found to be necessary for any of the subjects. The nebulizer was then switched on and, while wearing a nose clip, each subject was instructed to breathe normally through the mouthpiece. After breathing 5 L of hypertonic saline-laden air, single recordings of FEV₁ were made at 30 and 90 seconds and at 3 minutes. FEV₁ estimations that were technically unsatisfactory because of coughing were repeated. If the FEV₁ fell by more than 20% from the new baseline FEV₁ value, then the test end point had been reached. If not, additional volumes of air were respired, and the same procedure was repeated. The volumes used were: 5, 10, 15, 20, 20, 25, 35, 40, 40, 50, and 50 L (body temperature and pressure and saturated with water vapor), which were administered in this sequence until a fall in FEV₁ of 20% or greater

TABLE II. FEV₁ and PD₂₀HS values on control study day and on treatment days with atropine, ipratropium bromide, and lignocaine

Patient No.	Before study		Atropine			Ipratropium bromide			Lidocaine		
	FEV ₁ (L)	PD ₂₀ HS (L)	FEV ₁ (L)		PD ₂₀ HS (L)	FEV ₁ (L)		PD ₂₀ HS (L)	FEV ₁ (L)		PD ₂₀ HS (L)
			Pre	Post*		Pre	Post*		Pre	Post†	
1	3.65	10	3.90	3.85	116	3.75	3.90	76	—	—	—
2	3.75	9	3.90	3.95	117	3.75	3.75	30	3.60	3.80	33
3	3.75	36	3.65	4.10	620	3.45	4.35	443	3.65	3.50	477
4	2.70	60	2.25	2.40	51	1.45	1.80	37	1.75	1.65	76
5	2.70	219	2.80	2.80	315	2.90	2.90	620	2.65	2.40	361
6	3.85	290	3.85	3.90	285	3.85	3.80	255	3.70	3.45	283
7	4.30	139	4.25	4.30	620	4.00	4.00	620	4.00	3.85	226
8	3.30	75	3.10	3.20	193	3.20	3.25	170	2.80	2.95	202
9	6.05	270	5.90	6.20	344	6.70	7.20	443	5.55	4.55	564
10	3.30	30	3.65	4.20	413	2.50	2.15	132	3.70	3.05	—
11	3.65	125	3.70	3.55	131	4.50	4.50	56	4.50	4.60	460
Mean ± SEM	3.72 ± 0.27	115 ± 31	3.72 ± 0.27	3.85 ± 0.29	291 ± 59	3.64 ± 0.39	3.78 ± 0.42	230 ± 69	3.59 ± 0.32	3.38 ± 0.28	298 ± 58

The PD₂₀HS values shown are calculated with a top value of 620 L when the fall in FEV₁ was too small to permit linear extrapolation.

Pre, baseline FEV₁ before drug treatment; Post*, FEV₁ at 30 minutes after drug treatment; Post†, FEV₁ at 10 minutes after drug treatment.

had occurred or a total volume of 310 L of hypertonic saline-laden air had been respired.

The dose of hypertonic saline administered was expressed as the volume of air drawn from the nebulizer and plotted on a linear scale against the percentage fall in FEV₁. The PD₂₀HS was then derived by linear interpolation of the last two points, or when not possible, by linear extrapolation. If the fall in FEV₁ was too small to permit extrapolation, then the PD₂₀HS was estimated to be 620 L.

Repeatability of PD₂₀HS estimations. The PD₂₀HS estimations made on entry to the study were compared with those performed at the end of the study without any prior drug treatment in six subjects. The initial mean PD₂₀HS values 109 (±44) were not significantly different from later PD₂₀HS values 113 (±47) ($p = 0.8$), and the coefficient of variation for the hypertonic challenge on two visits at an interval of 1 week was 11.71%. The repeatability was assessed in the six subjects to confirm the finding of our previous study in which we demonstrated the repeatability of the challenge.¹⁶ Because some subjects were not available for follow-up tests, repeatability could not be performed in all subjects.

Data analysis. Baseline FEV₁ measurements on the control study day and 3 treatment days were compared by means of two-way analysis of variance. Student's paired t test was used to assess significant change in FEV₁ after administration of atropine, ipratropium bromide, or lidocaine. Spearman's rank correlation was used to assess the effect of a change in baseline FEV₁ (Δ FEV₁) with each drug treatment on the change in

airway responsiveness to hypertonic saline (Δ PD₂₀HS).

The effect of drugs on PD₂₀HS was analyzed with Wilcoxon's paired signed-rank test, because the absolute PD₂₀HS values could not be obtained in some subjects on some study days. The effects of age, sex, use of inhaled corticosteroids, baseline FEV₁, and PC₂₀ histamine on airway responsiveness to hypertonic saline on each drug treatment day were determined by multiple regression analysis. The log values of PD₂₀HS and PC₂₀ histamine were used for regression analysis. The relationship between the effect of the anticholinergic drugs atropine and ipratropium bromide on PD₂₀HS was examined by use of Spearman's rank correlation (r_s). A p value of less than 0.05 was considered significant.

RESULTS

All but two subjects completed the study: one (no. 10) experienced symptomatic wheeze during inhalation of lidocaine, and therefore hypertonic saline challenge was not given; and the other (no. 1) could not attend the laboratory on the lidocaine treatment day because of an exacerbation of asthma.

The mean baseline FEV₁ for the group was 3.65 ± 0.301 , 94.6% (range, 77% to 125%) of the predicted value. Their geometric mean PC₂₀ histamine was 1.32 mg/ml (range, 0.04 to 13.7 mg/ml) (Table I). Baseline FEV₁ values did not differ significantly between any of the study days, the

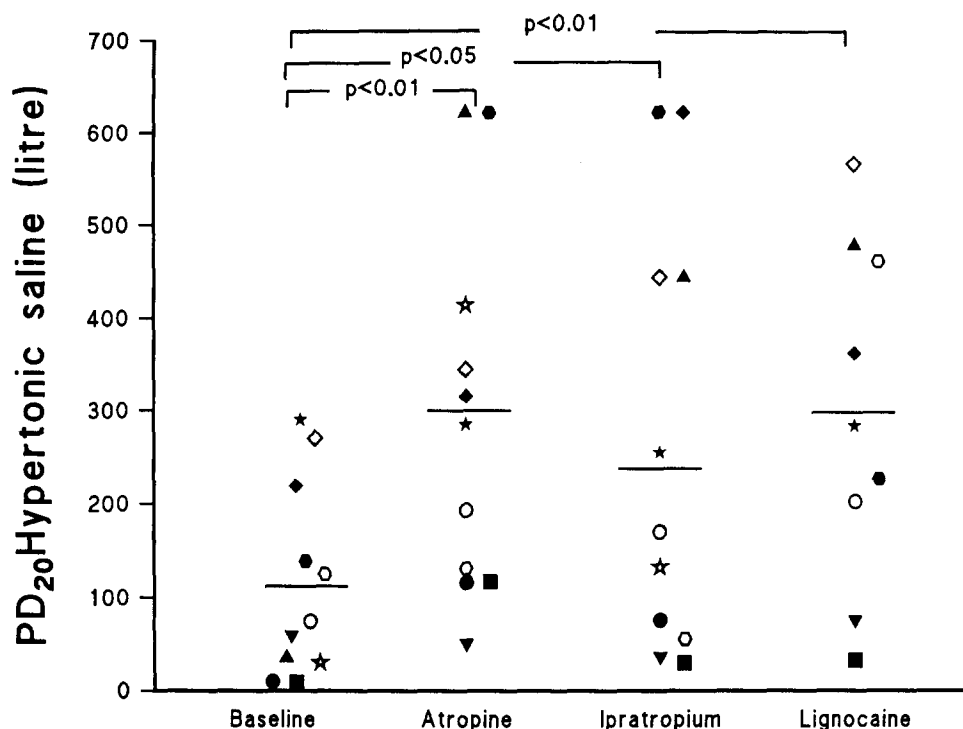


FIG. 1. PD₂₀HS values on control study day (baseline) and on treatment days with atropine, ipratropium bromide, and lidocaine.

mean values being $3.72 (\pm 0.27)$ on the control study day and $3.72 (\pm 0.27)$, $3.64 (\pm 0.39)$, and $3.59 (\pm 0.32)$ on treatment days with atropine, ipratropium bromide, and lidocaine, respectively (Table II). After administration of atropine and ipratropium bromide the mean FEV₁ values increased by 3.5% (± 1.6) and 4% (± 2.9) respectively (NS). After lidocaine administration, the mean FEV₁ decreased by 5.8% (± 1.5) from baseline (NS). In two subjects the decrease in FEV₁ was greater than 20% (Table II).

All of the drugs protected the airways against the constrictor effects of hypertonic saline. For atropine mean PD₂₀HS increased 2.5 times to 291 ± 59 from 115 ± 31 on the control day ($p = 0.009$). Similarly, with ipratropium bromide there was a twofold increase in mean PD₂₀HS to 230 ± 69 ($p = 0.04$). Lidocaine also afforded significant protection against hypertonic saline-induced bronchoconstriction, increasing the mean PD₂₀HS 2.6 times to 298 ± 57.1 ($p = 0.01$) (Fig. 1). There was no correlation between Δ FEV₁ and Δ PD₂₀HS on any of the drug treatment days (atropine $r_s = 0.31$, $p = 0.17$; ipratropium $r_s = 0.14$, $p = 0.33$; lidocaine $r_s = 0.39$, $p = 0.14$). The protection provided by each of the drugs was variable among individual subjects. With atropine it varied from no protection in three subjects

(Nos. 4, 6, and 11) to maximum increase of 17.2 times. Similar results were observed for the ipratropium, showing no increase in the same three subjects to a maximum increase of 12.3 times the baseline value. There was a good correlation between protection afforded by the two antimuscarinic drugs ($r_s = 0.82$, $p = 0.001$). With lidocaine there was a no increase in protection in two subjects (Nos. 3 and 4) to a maximum increase of 13.2 times. We failed to observe any effect of the age, sex, use of inhaled corticosteroids, baseline FEV₁, or PC₂₀ histamine on the PD₂₀HS response on any of the drug treatment days.

DISCUSSION

Atropine, ipratropium bromide, and lidocaine are used as premedication for bronchoscopy in patients with asthma to study the effect of a local airway challenge. The results of this study suggest that these drugs, given in the same route, dose, and interval as used for the premedication in bronchoscopy, could modify the response to local airway challenge and thus confound the interpretation of results of the challenge. This possibility has been ignored by investigators studying the effect of local airway challenge in asthma. In the present study we have shown that the antimusca-

rinic drugs atropine and ipratropium and the local anesthetic lidocaine protect against hypertonic saline-induced bronchoconstriction in asthma. All three drugs provided a similar degree of protection; however, there was a wide variation in response among subjects.

In this study atropine was given intramuscularly, because this route of delivery has been found to have a significant effect on efficacy.^{17, 18} Chen et al.¹⁷ reported that increasing the dose of inhaled atropine had no effect on EIA but that intramuscularly administered atropine protected against EIA. Similarly, Sheppard et al.^{18, 19} demonstrated that a more variable and much higher dose of inhaled atropine were required to protect against cold air-induced bronchoconstriction, when a smaller dose given intravenously was uniformly effective. Furthermore, atropine is commonly given via the intramuscular route as premedication for bronchoscopy.

Dose-response studies with inhaled ipratropium have shown that a dose of 50 to 125 μ g provides optimal bronchodilator effect in asthma.²⁰ However, there is no consensus regarding the dose of the antimuscarinic drugs required to protect against exercise-, hyperventilation-, or cold air-induced asthma. Although it has been suggested that higher doses tend to show better protection,¹⁹ other studies have failed to show any dose-response effect.²¹ In the present study a high dose (500 μ g) of inhaled ipratropium was used. Similarly, there is no consensus on the protective effect of inhaled lignocaine in EIA; some studies have reported protection, and others have failed to confirm it.⁹⁻¹² A dose-response study with inhaled lidocaine by Weiss and Patwardhan²² demonstrated no significant difference in the effect of two doses (40 and 100 mg) on FEV₁.

In this study we did not observe any significant bronchodilation with the administration of antimuscarinic drugs. This was probably due to the near normal baseline airway size of the subjects in our study group. However, the effect of any change in airway calibre with drug treatment on the protection provided has been carefully excluded: there was no correlation between change in FEV₁ and protection afforded by the drug. Furthermore, the fact that antimuscarinic drugs and lidocaine, which have the opposite effect on airway size, provided almost an equal degree of protection suggests that change in airway size with the drug had no effect on protection. Previously, it has been shown that the protection afforded by antimuscarinic drugs is not

due to their effect on baseline airway size. Sheppard et al.¹³ demonstrated that although two different doses of inhaled atropine caused the same degree of bronchoconstriction, only the higher dose was effective in providing protection. Similarly, Chen et al.¹⁷ have shown that the dose of inhaled atropine causing maximal bronchodilation failed to protect against EIA.

Previous studies have demonstrated a large individual variability in the effect of antimuscarinic drugs and lidocaine in EIA.^{9-12, 20} We observed a similar response with atropine, ipratropium, and lidocaine in hypertonic saline-induced bronchoconstriction. Although the reason for the variability is not clear, other authors have attributed it to individual differences in the relative importance of vagal mechanisms and mediator release²³ and differences in the sites of bronchoconstriction in individual subjects.²⁴ In this study no significant relationship could be established between the effect of drug treatment on PD₂₀HS and the subjects' age, sex, use of inhaled corticosteroids, FEV₁ or PC₂₀ histamine.

The mechanisms of hypertonic saline-induced bronchoconstriction are not clearly understood. The suggested mechanisms include mediator release from mucosal mast cells and stimulation of the neurogenic reflexes.³ The activation of the primed mast cells for mediator release is a possibility because the selective H₁-receptor antagonist, terfenadine, has been shown to attenuate hypertonic saline-induced bronchoconstriction.¹⁶ However, the aforementioned attenuation was not complete, and terfenadine produced bronchodilation, raising the possibility that increase in the airway calibre could have been partly responsible for the protective effect.

Support for neurogenic mechanisms has been provided by studies demonstrating that hypertonic saline is a potent stimulus for inducing cough²⁵ and neurogenic inflammation in the eye.⁵ Other authors have reported a hypertonic saline-induced increase in vascular permeability in the rat trachea in a dose-dependent manner.⁶ The increase in permeability was potentiated by phosphoramidon, a neutral endopeptidase inhibitor, suggesting that hypertonic saline induces the release of neuropeptides. In addition, atropine has been shown to inhibit hypertonic saline-induced peripheral bronchoconstriction in dogs.⁸

In this study we have provided support for the role of neurogenic reflex stimulation in hypertonic saline-induced bronchoconstriction. It is likely that atropine and ipratropium provide protection

by inhibiting vagal efferent discharge in response to stimulation. Lidocaine afforded a similar degree of protection against hypertonic saline-induced bronchoconstriction. The exact mechanisms by which lidocaine affords protection against hypertonic saline-induced bronchoconstriction are not clear. Because lidocaine inhibits cough induced by nonisotonic aerosols,¹³ it may inhibit stimulation of the sensory nerves in the airways. It is also possible that lidocaine inhibits neuropeptide release from the afferent nerves. Lidocaine is likely to have effect on efferent pathway because bupivacaine has been shown to block nonmyelinated efferent parasympathetic fibers in dogs.²⁶

In conclusion, this study has provided the evidence for an important contribution of neurogenic reflexes in hypertonic saline-induced bronchoconstriction. As in the case of bronchoconstriction provoked by isocapnic hyperventilation and exercise more than one mechanism may be responsible for hypertonic saline-induced bronchoconstriction.

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