

The protective effect of nedocromil sodium and other drugs on airway narrowing provoked by hyperosmolar stimuli: A role for the airway epithelium?

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The airways of persons with asthma are sensitive to acute changes in airway osmolarity and to dehydration. In reviewing the literature it is clear that airway narrowing provoked by these stimuli is blocked acutely by inhaling aerosols of nedocromil sodium, cromolyn sodium, frusemide, bumetanide, and antihistamines and by chronic use of aerosol corticosteroids. The responses are unaffected by inhalation of amiloride and verapamil. We have previously proposed that increases in the osmolarity of airway surface liquid (ASL) occur as a result of the water lost by evaporation during hyperpnea with dry air. An increase or decrease in osmolarity of the ASL will also occur with deposition of hyperosmolar and hypoosmolar droplets. Changes in osmolarity of the ASL result in the movement of water out of (shrinkage) and into (swelling) the epithelial cell, and this necessitates regulatory volume increase or decrease by the cell. We propose that nedocromil sodium and cromolyn sodium can affect water transport into and out of the epithelial cell by an action on chloride ion channels. A unifying hypothesis to explain the protective effect of these drugs may be their capacity to affect regulatory volume increase or decrease in a variety of cell types. (J Allergy Clin Immunol 1996;98:S124-34.)

Key words: Asthma, aerosols, hyperosmolar saline, exercise, hyperventilation, nedocromil sodium, cromolyn sodium, diuretics, indomethacin, steroids

In 1981 we reported that aerosols of hyperosmolar saline caused the airways of persons with asthma to narrow.¹ This response was effectively inhibited by administering 20 mg of cromolyn sodium by nebulization.²

For some years we investigated the use of a hyperosmolar challenge as a bronchial provocation test for asthma and developed a standardized protocol.³⁻⁵ We also investigated the acute and chronic effects of drugs used in the treatment of asthma on the response to hyperosmolar saline.⁶⁻¹³ We found that nedocromil sodium,⁸ cromolyn so-

Abbreviation used:

ASL: Airway surface liquid

dium,^{2, 13} frusemide, and corticosteroids^{9, 13} were effective in shifting the dose-response curve to hyperosmolar saline. These same drugs have also been found to be effective against challenge by exercise, hyperventilation, and distilled water.¹⁴⁻¹⁹ We found that amiloride was ineffective in the majority of subjects with asthma who underwent challenge with hyperventilation with dry air¹⁴ and hyperosmolar saline.⁶

We have previously proposed that exercise and hyperventilation cause narrowing of the airways by means of a hyperosmolar stimulus. Initially we considered the possibility that the hyperosmolar stimulus occurred as a result of an increase in the ion concentration in the airway surface liquid.^{20, 21} Later we suggested that the epithelial cells may

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become dehydrated during exercise.²² More recently we have proposed that there may also be a small increase in the osmolarity of the airway submucosa/subepithelium as a result of water loss from the epithelial cells.²³ These same events would occur in the airways when a hyperosmolar stimulus was given directly by an aerosol.

We have made some interesting observations that suggest to us that, in addition to their recognized sites of action on mast cells and nerves, drugs that act to prevent the airways narrowing in response to evaporative water loss and hyperosmolarity may do so by an effect on epithelial ion regulation and water transport (Fig. 1). In light of our results it appeared that epithelial cell volume regulation (Fig. 2) and defending of the submucosa/subepithelium from volume loss in response to hyperosmolarity may be important but hitherto overlooked actions of some of these drugs. We considered the possibility that the loss of epithelial cell volume and its subsequent replacement by water from the submucosa/subepithelium may be important processes associated with events that subsequently caused the airways to narrow.²³

This article reports the results that we obtained, particularly in relation to the 4.5% saline challenge, a known hyperosmolar stimulus. We discuss our findings in relation to the possibility that these drugs, in addition to their well-described actions, may also act to protect against challenge with hyperventilation and hyperpnea by reducing epithelial volume loss by their action on Cl^- ion channels and the transport of water across the airway epithelium.

SUBJECTS

The subjects were recruited either from the Respiratory Investigation Unit at Royal Prince Alfred Hospital, Sydney, where they were undergoing routine assessment of their asthma, or from the University of Sydney. Most subjects with asthma experienced asthma symptoms during or after exercise.

Clinically stable subjects with asthma ranging in age from 18 to 56 years participated in these studies. Subjects were included if they demonstrated a 20% fall in FEV_1 after inhaling 4.5% saline (PD_{20}) or if they demonstrated a 20% fall in FEV_1 after inhaling increasing concentrations of saline, 1.8%, 3.6%, 7.2%, or 14.4% (PC_{20}) each for 3 minutes, as in the clemastine study.⁷ β_2 -Adrenoceptor agonists and cromolyn sodium were withheld for at least 6 hours, ipratropium bromide for 8 hours, and theophylline for more than 12 hours

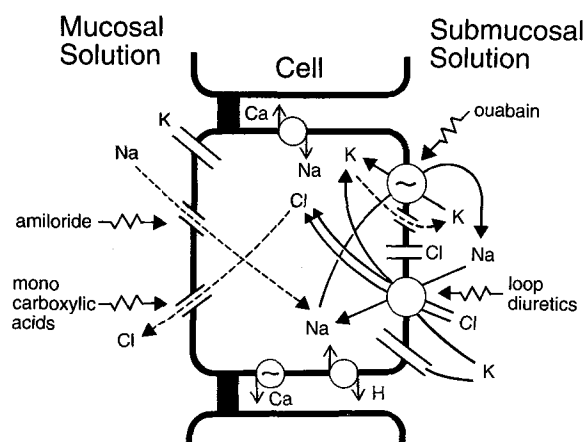


FIG. 1. Model of electrolyte transport in tracheal epithelium based on work in canine and human airways. The main features of the model are Cl^- , Na^+ , and K^+ channels, shown as *open hatches*; pumps Na^+K^+ adenosine triphosphatase and Ca^{++} , shown as *open circles with ~*, and the cotransport system for Na^+ , K^+ , and Cl^- and the antiport system Na^+H^+ and $\text{Ca}^{++}\text{Na}^+$, both shown as *circles with arrows*. Although the model depicts the Cl^- channel on the apical membrane as a single entity, it is likely to comprise three or four subtypes, one activated by protein kinase A and C and inhibited by Diphenylamine 2-Carboxylate, a second channel activated by raised intracellular Ca^{++} and inhibited by 4,4'-Diisothiocyano-2,2'-Disulphonic Acid Stilbene, and possibly a third channel activated by external adenosine triphosphate (but this may not be distinct from the second channel). A volume-sensitive Cl^- channel is localized on the basolateral membrane but may also be present on the apical surface. Under resting conditions there is Na^+ absorption and no Cl^- ion secretion. Cl^- ion secretion can be stimulated by β_2 -adrenoceptor agonist, adenosine, histamine, bradykinin, prostaglandin E_1 , leukotriene C_4 , and 15-HETE in amiloride-treated epithelia. Steroids are thought to increase the number of Na^+K^+ adenosine triphosphatase pumps but this is not proven for the airway. Water transport to the airway lumen would be affected by any disturbance of these ion transport systems but by Cl^- ion transport in particular. Under hyperosmolar conditions water moves from the cells to the lumen and this leads to cell shrinkage. Na^+ absorption is decreased as a consequence of deactivation of basolateral K^+ channels. The paracellular pathway becomes less permeable and the apical Cl^- permeability is decreased. Nedocromil sodium and cromolyn sodium may act to block the Cl^- channels at either the apical or basolateral surface, thus affecting cell volume regulation in response to osmotic changes. (Modified from Jacobs ER, De Coursey TE. Ion channels in the metabolic regulation of respiratory cells. Clin Chest Med 1989;10:25-36, in accordance with reference 43.)

before hyperosmolar saline challenge. Many subjects were atopic and were taking inhaled corticosteroids every day in doses ranging from 100 to 2400 μg . The dose of inhaled corticosteroid re-

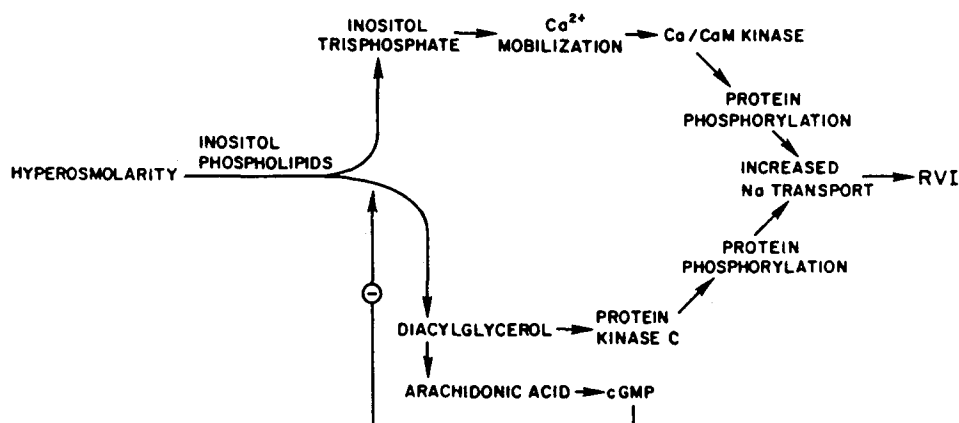


FIG. 2. Action of phosphoinositide pathway, protein kinase C, and Ca^{2+} on activating cell transport systems during volume regulation. (From Eveloff JL, Warnock DG. Activation of ion transport systems during cell volume regulation. *Am J Physiol* 1987;252:F1-F10.)

remained the same throughout the entire duration of each study. Subjects were asked about recent symptoms of wheeze or dyspnea. If they had experienced an increase in symptoms in the previous 24 hours, testing did not proceed. Each protocol was explained in detail, and consent was obtained in writing from all subjects. The studies were approved by the Ethics Committee at Royal Prince Alfred Hospital. For the majority of studies, the predicted lung function values were taken from Goldman and Becklake.²⁴ For the clemastine study, predicted lung function values were taken from Crapo et al.²⁵

DELIVERY OF AEROSOLS

Active and vehicle

Nedocromil sodium, cromolyn sodium, frusemide, bumetanide, and amiloride were all delivered from a Fisoneb (Fisons plc, Loughborough, United Kingdom) by means of a mouthpiece. This ultrasonic nebulizer, with an operating frequency of 1.6 MHz, produces a particle size with a mass median aerodynamic diameter of 4.5 to 5.0 μm ²⁶ (Malvern Mastersizer, Malvern Instruments Ltd., Worcestershire, United Kingdom). The unit was weighed on a balance (Sartorius AG, Göttingen, Germany) before and after nebulization to calculate the delivered dose.

The clemastine was delivered from an Up-draft 1700 jet nebulizer (Hudson RCI, Temecula, Calif.) and the verapamil from a Cadema jet nebulizer (Cadema Medical Products Inc., Middletown, N.Y.). The nebulizers were driven by compressed air at a flow rate of 6 to 8 L/min, producing a mass median aerodynamic diameter of 3.5 μm for the

particles generated by the Up-draft nebulizer²⁷ and 2.3 μm for those generated by the Cadema nebulizer.²⁸

HYPEROSMOLAR AEROSOL CHALLENGE

Hyperosmolar aerosols used for all challenges were generated by a MistO₂gen Ultrasonic Nebulizer (Timeter Instruments Corp., St. Louis, Mo.) Subjects inhaled the aerosols at resting ventilation rates through a two-way valve (Hans Rudolph 2700, Hans Rudolph, Inc., Kansas City, Mo.) connected to the nebulizer by Bennetts tubing (Bennett Respiration Products, Los Angeles, Calif.) (cat. No. TV 2723), 67.5 cm in length, with a smooth-bore internal diameter of 2.2 cm. This unit was weighed with the tubing but not the valve before the bronchial challenge and after the final dose of 4.5% NaCl had been delivered, or before and after each concentration of hyperosmolar saline solution had been delivered. Thus the output of the nebulizer over time was known for each subject on each occasion.

The protocol used to perform the challenge with 4.5% NaCl is described in detail by Smith and Anderson³ and has been slightly modified by Rodwell et al.⁸ The challenge was stopped when a fall in FEV₁ equal to or greater than 20% was recorded or the maximum dose or concentration of aerosol was delivered.

STUDY DESIGN

Each subject was usually required to visit the laboratory on a minimum of three occasions. On visit one, the control day, subjects were accepted

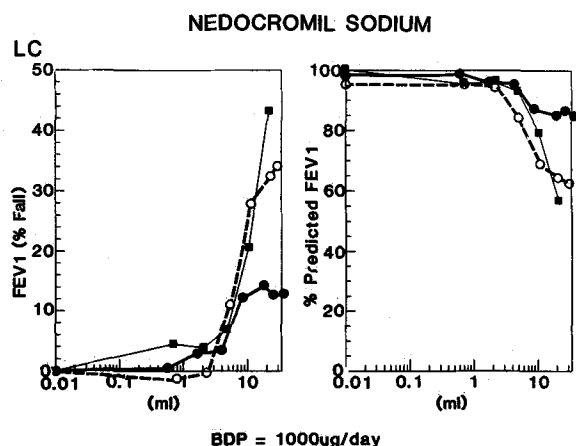


FIG. 3. Relationship between the fall in FEV_1 , expressed as a percentage of the prechallenge value (% fall) and a percentage of the predicted normal value (% predicted), and the cumulative dose of 4.5% saline aerosol delivered to the inspiratory port of a Hans Rudolph 2700 valve. The challenges were performed on three separate days: a control day (■), vehicle day (○), and after 10 mg of nedocromil sodium given by nebulization (●). This figure is typical of the finding, in eight of 16 subjects, of a plateau in response to 4.5% saline solution in the presence of nedocromil sodium. The subject was taking 1000 μ g of beclomethasone (BDP) per day. In the other eight subjects nedocromil sodium caused a significant shift in the dose-response curve but no plateau was observed. (From Rodwell LT, Anderson SD, du Toit JI, Seale JP. Nedocromil sodium inhibits the airway response to hyperosmolar challenge in patients with asthma. *Am Rev Respir Dis* 1992;146:1149-55.)

into the study if they had a PD_{20} FEV_1 to a hyperosmolar saline challenge. They returned for visits two and three to receive the active drug or its vehicle. It was usual for 3 days to separate each visit to ensure that the medication had cleared from the airways.

Control day

On arrival at the laboratory, FEV_1 was measured in triplicate and the baseline FEV_1 was taken as the best FEV_1 value, in liters, recorded immediately before hyperosmolar saline challenge.

Active drug and vehicle day

The protocol for the active drug and vehicle day was the same as for the control day. In addition, subjects inhaled either the active drug or its vehicle, usually 10 or 15 minutes before a hyperosmolar saline challenge. The challenge was stopped when either a 20% fall in FEV_1 was recorded or the maximum dose or concentration of aerosol was delivered.

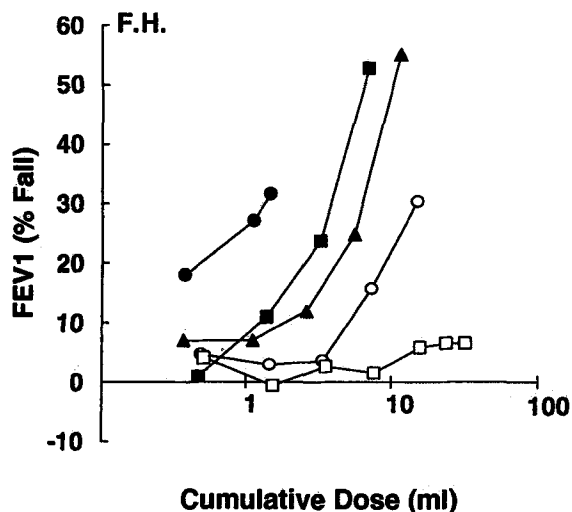


FIG. 4. Relationship between the fall in FEV_1 , expressed as a percentage of the prechallenge value (% fall), and the cumulative dose of 4.5% saline aerosol delivered to the inspiratory port of a Hans Rudolph 2700 valve. The subjects remained responsive to 4.5% saline solution after 4 (■) and 12 (▲) weeks of treatment with budesonide (1000 μ g/day). This figure illustrates the beneficial effect of the acute administration of 20 mg of cromolyn sodium by nebulization, both alone (○) and after 8 weeks of treatment with budesonide (□). (From Anderson SD, du Toit JI, Rodwell LT, Jenkins CR. Acute effect of sodium cromoglycate on airway narrowing induced by 4.5 percent saline aerosol: outcome before and during treatment with aerosol corticosteroids in patients with asthma. *Chest* 1994;105:673-80.)

Airway sensitivity (PD_{20} or PC_{20})

Airway sensitivity was measured as the provoking dose or concentration of hyperosmolar saline that caused a 20% fall in FEV_1 (PD_{20} or PC_{20}). These values were calculated by linear interpolation with use of the cumulative dose of 4.5% NaCl or the concentration of hyperosmolar saline causing a 20% fall in FEV_1 from the prechallenge value. The cumulative dose data was $\log_{10}$ transformed and parametric statistics were performed on the $\log_{10}$ transformed data.

Changes in PD_{20} or comparisons of the maximum percent fall in FEV_1 at the highest concentration of saline delivered that were common to both the active and vehicle day for each subject were used to assess the effects of the drug on airway sensitivity to hyperosmolar saline challenge. Where a 20% fall in FEV_1 was not recorded, the maximum dose or concentration of hyperosmolar saline delivered on the challenge day was used as the PD_{20} or PC_{20} .

When comparing the airway response on two challenge days, the mean change in the PD_{20} to

Table I. Summary of drug effects on responses to hyperosmolar saline challenge

Drug	n	FEV ₁ % predicted posttreatment (mean ± 1 SD) V or C	FEV ₁ % predicted posttreatment (mean ± 1 SD) active	PD ₂₀ geometric mean (ml) (95% CI) control	PD ₂₀ geometric mean (ml) (95% CI) vehicle	PD ₂₀ geometric mean (ml) (95% CI) active	p
Nedocromil sodium	16	90.6 ± 14	90.4 ± 16	2.88 (1.6, 5.3)	5.6 (3.3, 9.7)	22.2 (15.1, 32.7)	<0.001
Cromolyn sodium	11	83.5 ± 21	82.7 ± 21	2.76 (1.4, 5.4)	—	22.3 (13, 38)	<0.0003
Budesonide	11	83.5 ± 21	90.8 ± 14	2.76 (1.4, 5.4)	—	16.7 (7.1, 39)	<0.0003
Budesonide and cromolyn sodium	11	83.5 ± 21	91.4 ± 17	2.76 (1.4, 5.4)	—	44.1 (30.2, 64.3)	<0.0002
Beclomethasone	13	77.3 ± 12	85.4 ± 13	1.16 (0.5, 2.8)	—	6.5 (3.4, 12.3)	<0.005
Frusemide I	11	85.3 ± 20	85.4 ± 15	1.97 (1.2, 3.4)	1.32 (0.7, 2.3)	8.2 (4.7, 14.1)	<0.001
Frusemide II	14	81.5 ± 13	84.7 ± 13	4.2 (2.5, 7.1)	4.9 (2.5, 9.6)	16.3 (11.2, 24)	<0.0003
Frusemide II and indomethacin	14	81.5 ± 13	83.3 ± 12	4.2 (2.5, 7.1)	5.4 (2.9, 9.9)	20.2 (14.5, 28.4)	<0.0008
Indomethacin	14	81.5 ± 13	79.4 ± 13	4.2 (2.5, 7.1)	4.9 (2.5, 9.6)	5.4 (2.9, 9.9)	NS
Frusemide III	12	90.6 ± 10	86.3 ± 10	2.2 (0.8, 6.5)	—	27.1 (17, 43)	<0.001
Bumetanide	12	90.6 ± 10	86.5 ± 14	2.2 (0.8, 6.5)	—	11.4 (7.8, 16.7)	<0.001
Amiloride	9	90.3 ± 12	78.9 ± 22	2.7 (0.8, 8.4)	—	6.5 (2.8, 15.5)	NS
Verapamil	9	88 ± 17	83 ± 19	—	42%*	37%*	NS
Clemastine	8	89.2 ± 15	91.8 ± 18	PC ₂₀	42%* 16.8	17%* 12.1	0.015

On the control day (C), no medication was given. The vehicle (V) was the carrier of the active drug and acted as its placebo.

*% Fall FEV₁ after challenge. All drugs, with the exception of indomethacin, were given as aerosols and, except for budesonide and beclomethasone, from a nebulizer.

4.5% NaCl was expressed as a fold difference ± 95% confidence intervals. The percent fall in FEV₁ recorded at the common concentration of hyperosmolar saline delivered was expressed as the mean ± 1 SD.

MAXIMAL RESPONSE PLATEAU

The shape of the dose-response curve was investigated for the presence of a maximal response plateau. A maximal response plateau was considered if the change in FEV₁, expressed as a percentage of the baseline, varied less than 5% at the highest two doses. The maximal percent fall in FEV₁ was then calculated by averaging these data points.²⁹ A complete block was recorded if a less than 6% fall in FEV₁ occurred after the maximum dose of 4.5% NaCl had been nebulized. The value of 6%

represented the mean percent fall in FEV₁ recorded in healthy subjects at the time of the study.⁵

RESULTS

Results for the various studies are summarized in Table I.^{6-13, 30} Nedocromil sodium, cromolyn sodium, budesonide, beclomethasone, frusemide, bumetanide, and clemastine all caused a significant inhibition of airway narrowing in response to the inhalation of hyperosmolar saline. Clemastine caused a significant decrease in the maximum percent fall in FEV₁ at the highest concentration of hyperosmolar saline common to both the active and vehicle days. Amiloride, indomethacin, and verapamil had no significant effect on the airway response to hyperosmolar saline. We made a num-

Table I. Cont'd

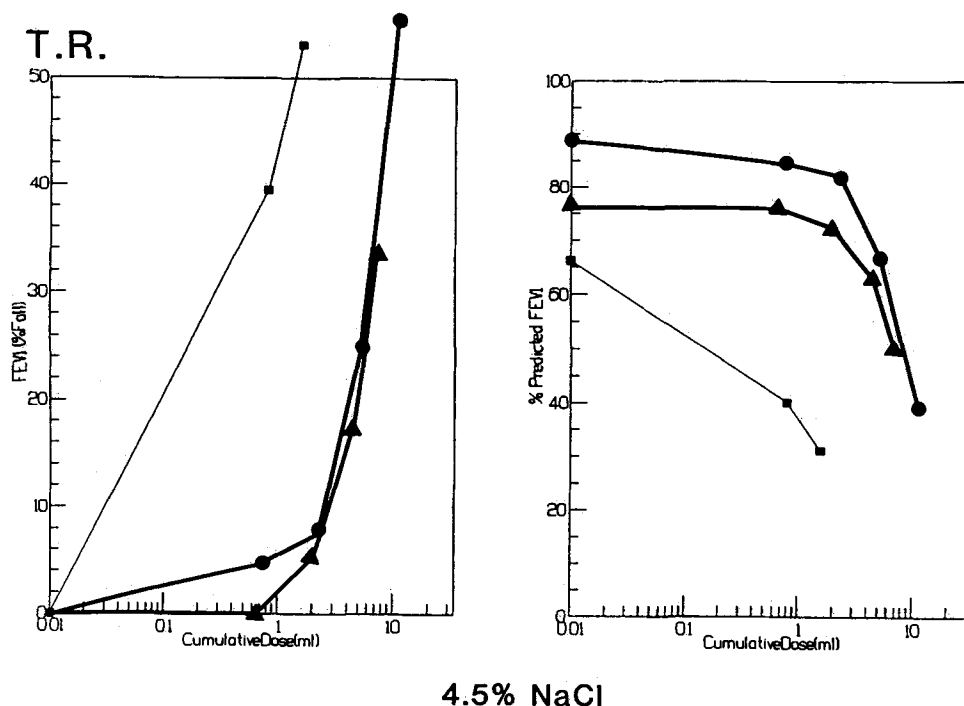
Fold difference	Delivered dose	Time before challenge	Reference No.
3.95 (2.8, 5.5)	10 mg	10 min	8
8 (4.2, 15.3)	20.7 mg $\pm$ 2.2	10 min	13
6.1 (2.9, 12.5)	1000 μ g daily	36 $\pm$ 9 days	13
16.2 (6.7, 39)	As above/+20 mg cromolyn sodium	As above/10 min	13
5.6 (2.7, 11.7)	600–1500 μ g daily	62 $\pm$ 5 days	9
6.2 (4.4, 8.7)	33.2 mg $\pm$ 3.8	10 min	10
3.3 (2.0, 5.4)	38 mg $\pm$ 6.4	10 min	11
3.7 (1.9, 7.2)	38 mg/25 mg b.i.d.	10 min/3 days	11
—	25 mg b.i.d.	3 days	11
12 (5.6, 25.9)	42 mg $\pm$ 6.3	10 min	12
5 (2.3, 11.1)	1.1 mg $\pm$ 0.13	10 min	12
1.9 (0.6, 5.8)	17.3 mg $\pm$ 2.9	10 min	6
0	3.5 mg $\pm$ 0.3	30 min	30
—	0.5 mg $\pm$ 0.1	15 min	7

ber of original observations, which are summarized as follows:

1. We observed a plateau in six of the 16 subjects who inhaled nedocromil sodium 10 minutes before challenge; this had not been previously observed (Fig. 3). Of these six subjects, four were taking either 100, 400, or 1000 μ g of beclomethasone dipropionate.⁸
2. We found that an acute dose of cromolyn sodium (20 mg) given by nebulization shifted the dose-response curve to the same extent as 3 to 8 weeks of treatment with budesonide ($r = 0.88$, $p < 0.01$). A further reduction in sensitivity to hyperosmolar challenge was found when cromolyn sodium was given in the presence of budesonide. Nine of 11 subjects recorded either a plateau or a complete block in response in the presence of cromolyn sodium alone or in combination with budesonide (Fig. 4) with only two

subjects remaining responsive to the hyperosmolar stimulus.¹³

3. By contrast, for the three studies on frusemide summarized in Table I, we found only one subject who had a plateau in his or her airway response to hyperosmolar saline in the presence of frusemide, and only three subjects had a complete block after inhaling frusemide.¹⁰⁻¹²
4. We observed that frusemide not only reduces airway sensitivity to hyperosmolar saline solution challenge but also causes a transient bronchodilation during challenge.¹⁰ This bronchodilation was not affected by giving subjects indomethacin or its vehicle for 3 days.¹¹
5. The antihistamine clemastine, when administered as an aerosol, was effective in reducing the maximum fall in FEV₁ at the concentration of saline common to both the vehicle and active days (42.2% $\pm$ 16.8 vs 17.0% $\pm$ 12.1; $p < 0.015$).⁷ Unfortunately the aerosol has a high osmolality itself, and thus it is not practical to use.
6. We were surprised to find that for subjects taking beclomethasone dipropionate, the majority remained responsive to hyperosmolar saline solution (Fig. 5), and only one of the 13 subjects had a negative response.⁹ By contrast, after 8 weeks of treatment with budesonide, 50% of subjects had a negative response to 4.5% NaCl challenge after treatment with 1000 μ g/day of budesonide^{13,31} (Fig. 6).
7. We found that bumetanide had a significant beneficial effect against 4.5% NaCl. After inhaling bumetanide there was a 5.1 (2.3-11.1) ($p < 0.001$) fold increase in the PD₂₀ from the control (Table I).¹²
8. Both bumetanide and frusemide, at an equipotent diuretic dose, significantly inhibited airway narrowing to 4.5% NaCl challenge in 12 subjects with asthma (Table I), which is in keeping with the concept that the Na⁺/K⁺/2Cl⁻ cotransporter could be involved in hyperosmolality-induced asthma. Frusemide was, however, more effective than bumetanide at inhibiting the airway response to 4.5% NaCl challenge as measured by the PD₂₀ (27.1 ml [17.0 to 43.3] vs 11.4 ml [7.8 to 16.7] [$p < 0.001$]). These findings suggest that frusemide may have an additional mechanism relating to its protective effect.^{6, 12, 32}
9. For the group of subjects with asthma who were studied, amiloride was ineffective at inhibiting airway responses to 4.5% NaCl challenge. In fact, in some subjects amiloride had a profound effect on baseline lung function. One subject



4.5% NaCl

FIG. 5. Relationship between the fall in FEV₁, expressed as a percentage of the prechallenge value (% fall) and a percentage of the predicted normal value (% predicted), and the cumulative dose of 4.5% saline aerosol delivered to the inspiratory port of a Hans Rudolph 2700 valve. The challenges were performed before taking beclomethasone dipropionate (■) and 7 (●) and 18 weeks (▲) later. This illustrates the pattern seen for 11 of 13 subjects who were taking beclomethasone in a dose of 600 to 1500 µg/day, that is, there was an improvement in baseline lung function in response to beclomethasone but the subject remains very responsive to hyperosmolar challenge. It should be noted that the concentration of the hyperosmolar saline aerosol was not increased, only the duration of the exposure to the aerosol. (From Rodwell LT, Anderson S, Seale JP. Inhaled steroids modify bronchial responses to hyperosmolar saline. *Eur Respir J* 1992;5:953-62.)

dropped from 66.9% to 27.4% of the predicted FEV₁ immediately after inhaling amiloride and was not able to proceed with the 4.5% NaCl challenge. However, three subjects with asthma had a 7.4-, 7.6-, and 11.5-fold increase in PD₂₀ FEV₁ to 4.5% NaCl challenge from the control day after inhaling the amiloride.⁶

DISCUSSION

The results of the studies presented here clearly demonstrate that many drugs reduce the sensitivity to hyperosmolar saline when given either acutely or chronically, as in the case of corticosteroids. This reduction in sensitivity occurred independently of change in baseline airway caliber. All but one patient remained responsive when taking beclomethasone, and half of those treated with budesonide remained responsive after 8 to 11 weeks of treatment. In addition to causing a marked reduction in sensitivity to 4.5% saline,

both nedocromil sodium and cromolyn sodium were able to limit the extent to which the airways narrowed, that is, in some subjects a plateau occurred in the airway response to the administration of 4.5% saline (Fig. 3). Although a plateau or total inhibition of the response was most likely to occur in persons who had moderate-to-mild sensitivity to 4.5% saline and who were taking aerosol steroids, this was not always the case. While most subjects who achieved a plateau in their response in the presence of nedocromil sodium were taking either beclomethasone or budesonide, others achieved this plateau in the absence of steroids. These observations have important implications for the treatment of asthma because one of the aims of treatment is to limit the extent to which the airways narrow in response to a stimulus.

In addition to inhibiting the airway narrowing in response to a known hyperosmolar stimulus, nedocromil sodium also inhibits airway narrowing

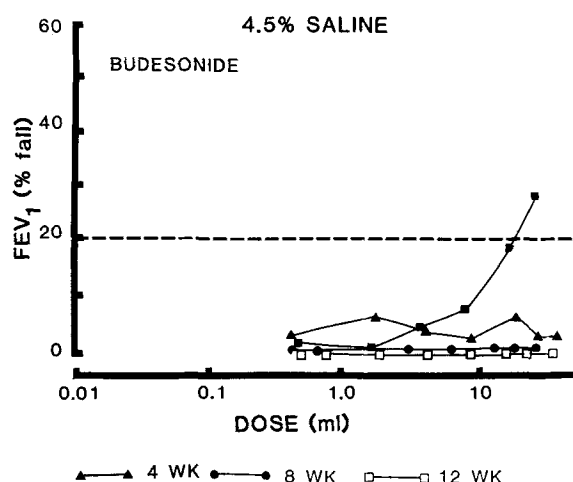


FIG. 6. Relationship between the fall in FEV_1 , expressed as a percentage of the prechallenge value (% fall) and the cumulative dose of 4.5% saline aerosol delivered to the inspiratory port of a Hans Rudolph 2700 valve. The airway response to saline observed on the control day (■) was inhibited by 4 (▲), 8 (●), and 12 (□) weeks of treatment with budesonide (1000 μ g/day). We have found that the response of 50% of patients to 4.5% saline solution is inhibited after 8 weeks of treatment with budesonide (1000 μ g/day). For the remaining 50% of the patients, the dose-response curve was markedly shifted but the subjects remained responsive to challenge with 4.5% saline solution.

in response to exercise-induced hyperpnea. A summary of some of the published data is given in Table II.^{16, 17, 33-40} These data repeatedly demonstrate a significant but incomplete protection by nedocromil sodium against the airway narrowing provoked by 5 to 8 minutes of vigorous running exercise both in adults and children. Extrapolating from the results of the use of hyperosmolar saline presented here, one could predict that, in subjects treated with steroids, nedocromil sodium may completely prevent the airway response to exercise.

There are two sites at which nedocromil sodium and cromolyn sodium have been traditionally thought to act to prevent airway narrowing in response to hyperpnea and hyperosmolarity. These sites include the mast cell and the sensory nerves. Data from human and animal studies both in vivo and in vitro demonstrate that both hyperpnea with dry air and hyperosmolarity can induce mast cell release of mediators, histamine in particular, and cause sensory nerves to release neuropeptides. In addition to these described actions, we believe that they may also act on epithelial cells and affect the

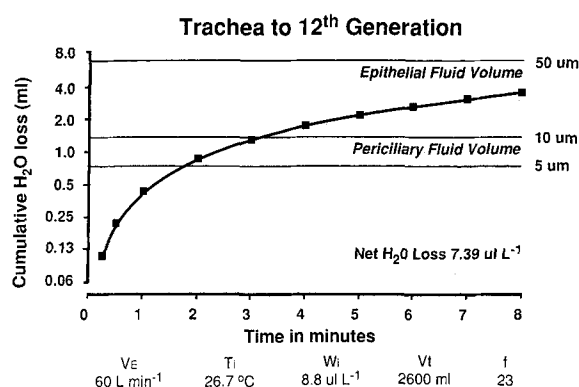


FIG. 7. Cumulative loss of water in relation to time of hyperpnea predicted by the mathematical model of Daviskas E, et al.⁴⁶ The horizontal lines intercept the axis at the cumulative volume of periciliary fluid available (assuming a depth of 5 or 10 μ m) and the cumulative volume of epithelial fluid available (assuming a cell depth of 50 μ m) from the trachea to the twelfth generation. For a ventilation rate of 60 L/min (a moderate exercise ventilation rate) and inspired air conditions of 26.7° C and 8.8 mg or μ L of H_2O per liter of air inspired (usual laboratory conditions), the net loss of water was calculated to be 7.39 mg or μ L H_2O per liter of inspired air. It was predicted that less than 2 minutes of hyperpnea would be required to dehydrate the periciliary fluid if it had a depth of 5 μ m, and 4 minutes would be required for a depth of 10 μ m. At more than 8 minutes of hyperpnea the fluid loss from the epithelial cells is predicted to be around 50% of volume. Thus to maintain normal hydration of the airways, water would need to be returned quickly to the periciliary fluid layer from the epithelial cell and returned quickly to the epithelial cell from the submucosa/subepithelium. The transport of water from the submucosa/subepithelium has the potential to cause small increases in osmolarity, signaling mediator release and an increase in bronchial blood flow in response to a hyperosmolar stimulus.²³ It is proposed that one site of action for nedocromil sodium and cromolyn sodium could be defending the submucosa/subepithelium from dehydration and osmotic changes by altering water transport by an action on ion channels of the epithelial cell. (From Anderson SD, Daviskas E, Smith CM. Exercise-induced asthma: a difference in opinion regarding the stimulus. *Allergy Proc* 1989;10:215-26.)

events that control epithelial cell volume regulation in response to osmotic stimuli (Fig. 2).

To explain the effect of these drugs on various cells in the airways, we considered that their action may have been in blocking the Cl^- ion channels. We have previously proposed that the regulation of Cl^- ion channels may be altered by inflammation and that the potency of nedocromil sodium and cromolyn sodium to inhibit responses to hyperosmolar stimulus may relate to their ability to block this channel.¹³

Table II. Summary of effects of nedocromil sodium on responses to exercise challenge

Reference No.	N	Group	Dose (mg)	Type of exercise	Min	% Fall (control)	% Fall (placebo)	% Fall (nedocromil)	p	Protection (%)
33	24	C	4, MDI	Running	6	29.8 ± 2.90	28.5 ± 2.9	15.4 ± 1.7	<0.001	58.3
34	12	C	4, MDI	Running	6	36.2 ± 4.1	31.3 ± 4.6	14.5 ± 3.7	<0.005	59.3 ± 9.0
17	16	A/C	8, MDI	Running (sub-max) 70% VO ₂	8	33.2 ± 5.7	38.4 ± 4.7	15.8 ± 3.5	<0.001	62.5
17	12	A	8 MDI	Running	8	—	34.2 ± 5.2	16.6 ± 3.4	<0.005	51.5 ± 9.1
35	10	A	0.5 mg/ml	Running	6–8	—	31.0 ± 2.3	12 ± 2.0	<0.001	61.6 ± 8.7
			2.5 mg/ml	(sub-max)				9 ± 2.2		71.0 ± 4.3
			4.0 mg/ml					8 ± 2.3		73.8 ± 5.6
36	20	C	4, MDI	Running	6	39.9 ± 2.4	22.7 ± 2.5	15.7 ± 2.5	<0.05	59.4
[37]*	12	A	4, MDI	Running	6	39.7 ± 4.0	44.2 ± 4.3	15.0 ± 3.6	<0.05	59.7 ± 8.7
16	12	A	4, MDI	Running	6	—	26.2 ± 3.7	12.0 ± 4.4	<0.05	63.0
[38]*	14	A/C	4, MDI	Running	8	39.9 ± 4.4	21.9 ± 4.2	13.2 ± 3.33	<0.01	
39	12	C	4, MDI	Running	6	≈40 ± 5	≈39.5 ± 5	≈21 ± 5	<0.001	≈50%
40	9	A	4, MDI	Running	5–8	40.6 ± 5.5	38.9 ± 6.3	18.9 ± 5.9	<0.001	
			2					13.9 ± 5.0		

A, Adults; C, children; MDI, metered dose inhaler.

*Reported peak expiratory flow rather than FEV₁.

This concept arose from the knowledge that Cl⁻ ion channels are blocked by monocarboxylic acids (Fig. 1) and that it was possible that dicarboxylic acids may act in the same way (Diana Temple, University of Sydney, personal communication). Both nedocromil sodium and cromolyn sodium are dicarboxylic acids. At the time, we were considering the Cl⁻ ion channel on the airway epithelial cell and its response to a hyperosmolar stimulus (Fig. 2). Later, we discovered that Romanin et al.⁴¹ had found that cromolyn sodium blocked the Cl⁻ ion channels on rat mast cells. We were excited by this finding because it was also likely that Cl⁻ ion channels on nerves could be blocked by these drugs⁴² and this effect could serve to explain the broad range of challenges blocked by nedocromil sodium and cromolyn sodium, for example, substance P, sulfur dioxide, and metabisulfite.

With hyperosmolarity of the airway surface liquid, water moves from the epithelial cell independently of Cl⁻ ion secretion and in response to an osmotic gradient.⁴³ However, in the presence of mediators (e.g., histamine, bradykinin, and prostaglandins) released in response to hyperosmolarity, Cl⁻ ion secretion could be stimulated. It may be that nedocromil sodium and cromolyn sodium alter this Cl⁻ ion channel activity under these circumstances (Fig. 1). By doing so these drugs may alter the rate of loss of water from the epithelial cell or the rate of water return to the epithelial cell from the submucosa. Preventing

excessive water loss from the epithelial cell would reduce the need for regulatory volume increase and the events associated with this, one being an osmotic change in the submucosa/subepithelium. We believe that the role of frusemide/bumetanide may be similar in their action in blocking Na⁺Cl⁻ cotransport on the basement membrane of the epithelial cell. Again, this action may be in addition to the capacity of these agents to inhibit mediator release.³²

In 1987 we proposed that the rate of water loss during hyperpnea was sufficiently high to cause dehydration of the epithelial cell²² (Fig. 7). In 1992 we proposed that replacement of the airway surface liquid and epithelial cell volume by water from the submucosa/subepithelium in response to an osmotic stimulus may cause a small but significant increase in osmolarity of the submucosa/subepithelium.²³ We proposed that this chain of events may be important in the airway narrowing caused by hyperosmolar stimuli. If the events of volume loss and regulatory volume increase of the epithelial cell (Fig. 2) could be modified, then this may prevent the airways narrowing in response to either evaporative water loss or a hyperosmolar stimulus. We believe that, in addition to their other well-described actions, drugs such as nedocromil sodium, cromolyn sodium, frusemide, bumetanide, and the corticosteroids may regulate epithelial cell volume changes and by doing so modify airway narrowing in response to osmotic stimuli. We

propose that nedocromil sodium and cromolyn sodium do this probably by blocking the movement of Cl^- ions into or out of the epithelial cell (Fig. 1). The results presented earlier at this symposium by Norris and Alton, showing that nedocromil sodium, cromolyn sodium, and frusemide are able to block the activity of a Cl^- ion channel on the mucosal surface of airway epithelial cells, support this concept. We believe that the action of frusemide and bumetanide is to reduce water transport to the epithelial cell by reducing Cl^- ion transport at the basolateral membrane of the epithelial cell. Our data demonstrating that frusemide delays the increase in mucociliary clearance after hyperpnea with dry air⁴⁴ is in keeping with this concept. We consider that steroids, in addition to their well-known antiinflammatory action, may reduce dehydration of the submucosa/subepithelium by increasing the number of $\text{Na}^+\text{K}^+\text{ATPase}$ pumps,⁴⁵ thus enhancing the flow of water towards the submucosa/subepithelium, preventing dehydration and changes in osmolarity. The potential role of β_2 -adrenoceptor agonists in this model would be to stimulate Cl^- ion secretion, thereby enhancing the movement of water to the airway lumen and potentially causing dehydration. This could explain why the protective effect of these drugs, particularly long-acting agents, is reduced with chronic use.

If nedocromil sodium and cromolyn sodium act to reduce the effects of dehydration, how would this affect the response to hyperosmolarity and hyperpnea? For hyperpnea, we would predict that the reduction in availability of water at the airway surface may cause more generations of airways to be recruited into the humidifying process. On one hand this is good, because it protects the submucosa/subepithelium of the large airways, where there is only a small volume of fluid, and diverts the air to be conditioned in the periphery where there is a much greater volume of fluid.²² On the other hand, recruiting the more peripheral airways could cause a greater bronchoconstrictor response if the loss of fluid by evaporation were sufficient to cause an increase in the ion concentration of the airway surface liquid. This may be the reason why these drugs do not completely prevent airway narrowing in response to hyperpnea. For the hyperosmolar aerosols, the stimulus may be confined to the first 10 to 12 generations as the capacity to change osmolarity significantly by aerosol particle deposition decreases as the airway surface liquid volume increases. This may be the reason for the plateau in response to nedocromil sodium.

We believe that the transport of water that occurs in response to a hyperosmolar stimulus in the airway has the potential to involve events that can be acutely blocked by drugs such as nedocromil sodium, cromolyn sodium, and the diuretics frusemide and bumetanide. These same drugs also inhibit airway narrowing in response to hypoosmolar stimuli where cell volume regulation is also important. We conclude that, in addition to their recently described inhibition of Cl^- channels on mast cells⁴¹ and possibly sensory nerves,⁴² nedocromil sodium and cromolyn sodium may reduce airway narrowing in response to hyperpnea and nonisotonic aerosols by an action on the events relating to water transport and regulation of cell volume that occur in response to these provocative stimuli. A unifying hypothesis to explain the protective effect of these drugs may be in their capacity to affect regulatory volume increase and decrease on a variety of cell types.

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