Relative potencies and time course of changes in adenosine 5'-monophosphate airway responsiveness with inhaled furosemide and bumetanide in asthma

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A randomized, double-blind, placebo-controlled study was conducted to compare the effects of two chemically unrelated "loop" diuretics, furosemide (40 mg) and bumetanide (2 mg) on the bronchoconstrictor response to inhaled adenosine 5'-monophosphate (AMP) in 12 subjects with asthma. In eight additional volunteers with asthma, we also carried out a separate randomized, double-blind study to examine in more detail the time course of change in bronchial reactivity to inhaled AMP after administration of nebulized furosemide and bumetanide. Inhaled loop diuretics significantly increased the provocative concentration of AMP causing a 20% fall in forced expiratory volume in 1 second (FEV_1) from the value of 21.2 mg/ml (range, 2.5 to 96.9 mg/ml) after placebo administration to 83.4 mg/ml (range, 11.3 to 345.0 mg/ml) ($p < 0.01$) and 33.8 mg/ml (range, 4.7 to 120.9 mg/ml) ($p < 0.05$) after administration of furosemide and bumetanide, respectively. After placebo administration, the provocative concentration of AMP causing a 20% fall in FEV_1 (PC_{20} AMP) at 10, 30, and 120 minutes did not differ significantly; their geometric mean (range) values were 57.8 mg/ml (10.9 to 341.0 mg/ml), 55.0 mg/ml (13.2 to 304.1 mg/ml), and 52.8 mg/ml (14.4 to 252.2 mg/ml), respectively. When compared with placebo, inhaled furosemide significantly reduced the airway responsiveness to AMP at all time points; the PC_{20} AMP values at 10, 30, and 120 minutes were 154.6 mg/ml (29.4 to 658.7 mg/ml) ($p < 0.01$), 142.6 mg/ml (25.5 to 639.9 mg/ml) ($p < 0.01$), and 103.9 mg/ml (12.5 to 605.5 mg/ml) ($p < 0.05$), respectively. The PC_{20} values for AMP after pretreatment with bumetanide were significantly increased up to 110.2 mg/ml (25.9 to 639.0 mg/ml) ($p < 0.01$) and to 92.0 mg/ml (21.6 to 531.7 mg/ml) ($p < 0.05$) at 10 and 30 minutes, respectively. At 120 minutes, inhaled bumetanide failed to affect AMP airway responsiveness; the PC_{20} AMP was not significantly different from that of placebo, with a value of 71.5 mg/ml (22.6 to 318.0 mg/ml). We conclude that comparable equidiuretic doses of furosemide and bumetanide are effective in attenuating the airway response to AMP, with furosemide being approximately 2.5 times more potent than bumetanide ($p < 0.01$). The time course of change in bronchial reactivity to AMP is similar for both drugs with a peak effect at 10 minutes. It is possible that the mechanism(s) underlying the protective effects of inhaled loop diuretics in asthma may be distinct from those responsible for their diuretic properties. (J Allergy Clin Immunol 1993;92:288-97.)

Key words: *Asthma, bronchoconstriction, adenosine, furosemide, bumetanide*

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Abbreviations used

AMP:	Adenosine 5'-monophosphate
ANOVA:	Analysis of variance
FEV_1:	Forced expiratory volume in 1 second
PC_{20}:	Provocative concentration causing a 20% fall in FEV_1
PG:	Prostaglandin

The "loop" diuretic, furosemide, when administered by inhalation has been shown to protect the asthmatic airways against various bronchoconstrictor stimuli such as allergen,[1] ultrasonically nebulized distilled water,[2] exercise,[3] cold air,[4] sodium metabisulfite,[5] methacholine,[6] and adenosine 5′-monophosphate (AMP).[6] "Loop" diuretics such as furosemide and bumetanide act by reducing salt reabsorption in the thick ascending limb of the loop of Henle[7] via the inhibition of the Na^+/K^+-adenosinetriphosphatase responsible for the Na-K-Cl cotransport across the tubular epithelium.[8, 9] When present at the basolateral side, "loop" diuretics inhibit Cl^- flux by a number of epithelia, possibly by an action on a linked Na^+/Cl^- entry process,[10] which supports the view that the drug may act by blocking the Na^+/K^+-adenosinetriphosphatase.

The mechanisms underlying the protective effects of these drugs in experimental models of clinical asthma are not yet understood. Although the inhibitory effects of inhaled furosemide in the airways in the absence of bronchodilatation might be explained by a suppressive action on airway mast cells, additional possibilities may include the capacity of this drug to locally generate protective prostaglandins with functional effects, to enhance bronchial blood flow and thus affect the kinetics of the clearance of bronchoconstrictor stimuli, and finally to inhibit neural pathways.[11]

AMP causes dose-related bronchoconstriction when inhaled by subjects with atopic[12] and nonatopic[13] asthma. Although the mode of action of adenosine in causing bronchoconstriction in asthma is not known with certainty, activation of inflammatory cells, particularly mast cells, is the most likely possibility.[14] Indeed, adenosine augments immunologically stimulated histamine release from rat peritoneal mast cells,[15] human lung mast cells,[16] and peripheral blood basophils[17] by a mechanism likely to involve A_2-purinoceptor stimulation. The enhancement of mast cell degranulation by adenosine in vitro is compatible with the observation that in atopic and nonatopic subjects with asthma the selective histamine H_1 receptor antagonists, terfenadine and astemizole, are able to almost totally inhibit bronchoconstriction induced by inhaled AMP.[13, 18] Enhancement of mast cell mediator release may not be the only mechanism that accounts for the bronchoconstriction provoked by inhaled AMP. In addition, local neural reflexes of both cholinergic[19] and noncholinergic origin[20] may also contribute to the response.

Having demonstrated that in a group of subjects with asthma inhaled furosemide was more effective in attenuating bronchoconstriction provoked by AMP than by methacholine,[6] we have now investigated whether this protective effect extends to another "loop" diuretic. Two chemically unrelated "loop" diuretics, furosemide, an anthranilic acid derivative, and bumetanide, a 3-aminobenzoic acid derivative, have been compared for their effects in protecting against AMP-induced bronchoconstriction. The study took the form of a randomized, double blind, placebo-controlled study of 12 subjects with asthma, with bumetanide being administered as an aerosol at one-twentieth the concentration of furosemide to ensure an adequate dose of bumetanide and to reflect their relative potencies as "loop" diuretics.[21]

Most studies have measured changes in airway response after administration of nebulized diuretics only at a single time point, and little is known of the change in bronchial reactivity with time. Because the timing of measurements may be important in comparative studies, we undertook a separate randomized, double-blind trial in eight additional subjects with asthma to examine in more detail the time course of change in bronchial reactivity to inhaled AMP after treatment with nebulized furosemide and bumetanide.

METHODS

Subjects

A total of 20 subjects with asthma (10 men) with a mean (±SEM) age of 42.2 years (±3.4 years), participated in the study (Table I). They were nonsmokers, and all except three were atopic as defined by positive skin prick test results (>2 mm wheal response) to two or more common aeroallergens (*Dermatophagoides pteronyssinus, D. farinae,* mixed grass pollen, mixed tree pollen, cat fur, dog hair, mixed feathers, *Aspergillus fumigatus, Candida albicans* [Bencard, Brentford, Middlesex, England]). All patients had baseline forced expiratory volume in 1 second (FEV_1) greater than 70% of their predicted values or greater than 2.0 L. None of them were receiving orally administered corticosteroids, theophylline, or sodium cromoglycate on a regular basis. Bronchodilators were withheld for 8 hours before each visit to the laboratory, although subjects were allowed to continue use of inhaled corticosteroids as usual. None of the subjects were studied within 4 weeks of an upper respiratory tract infection or exacerbation of their asthma. Subjects gave their written informed consent, and the study was approved by the Southampton University and Hospitals Ethical Committee.

TABLE I. Characteristics of subjects studied

Subject No.	Sex	Age (yr)	Baseline FEV_1 (% predicted)	Atopy*	PC_{20} methacholine (mg/ml)
1	F	25	96	+	0.86
2	M	65	91	−	0.38
3	M	36	95	+	0.52
4	M	44	88	−	0.60
5	F	52	80	+	1.21
6	M	27	82	+	0.43
7	M	44	72	+	0.31
8	M	52	75	+	0.44
9	F	30	123	+	1.42
10	F	24	95	+	1.15
11	F	29	85	+	0.11
12	F	18	81	+	0.08
13	M	57	87	+	
14	M	59	110	+	
15	M	64	117	−	
16	F	21	97	+	
17	F	40	129	+	
18	M	55	98	+	
19	F	54	121	+	
20	F	47	86	+	
Mean ± SEM		42.2 ± 3.4	92.9 ± 3.1		0.47† (0.08-1.42)

*Atopic, positive (+) immediate skin test to one or more allergens.
†Geometric mean (range).

Bronchial provocation

Pulmonary function was measured before and during the provocation as the FEV_1 with a dry wedge spirometer (Vitalograph, Buckinghamshire, England); the first of two consecutive measurements was used for analysis. Methacholine (Sigma Chemical Co., Poole, Dorset, England) and AMP (Sigma Chemical Co., St. Louis, Mo.) were made up in 0.9% sodium chloride to produce a range of increasing doubling concentrations of 0.03 to 64.00 mg/ml (0.2 to 327 mmol/L) and 0.78 to 800 mg/ml (8.96 to 1151.4 mmol/L), respectively. The solutions were administered as aerosols generated from a starting volume of 3 ml in a disposable Inspiron mini-nebulizer (C. R. Bard International, Sunderland, England) driven by compressed air at 8 L/min. Under these conditions, the nebulizer has an output of 0.48 ml/min and generates an aerosol with a mass median particle diameter of 4.7 μm.[22] While wearing a nose clip, each subject inhaled the aerosolized solutions via a mouthpiece as 5 breaths from end tidal volume to full inspiratory capacity.[23] All of the bronchial provocations were carried out at the same time of day.

Study design

The study consisted of two distinct phases. In the first phase, 12 patients with asthma (nos. 1 to 12) attended the laboratory on five separate occasions, at least 72 hours apart. On the first two visits subjects attended the laboratory to undertake concentration-response studies with inhaled methacholine and AMP in the absence of any drug treatment. On the first occasion, after a 15-minute rest, three baseline measurements of FEV_1 were made at intervals of 3 minutes followed by inhalation of 0.9% sodium chloride and repeat FEV_1 measurements at 1 and 3 minutes, and the higher value was recorded. Provided that the FEV_1 did not fall by more than 10% of the baseline value, a methacholine concentration-response study was carried out. After administration of each methacholine concentration, FEV_1 was measured at 1 and 3 minutes. Increasing doubling concentrations of methacholine were inhaled at 5-minute intervals until FEV_1 had fallen by more than 20% of the value after saline challenge. The fall in FEV_1 after each concentration of agonist was expressed as a percentage of the higher of the two baseline FEV_1 recordings after saline challenge. The percentage fall in FEV_1 was plotted against the cumulative concentration of agonist on a logarithmic scale, and the provocation concentration required to produce a 20% decrease in FEV_1 (PC_{20}) from the baseline value after saline challenge was determined by linear interpolation. On the second occasion, a bronchial provocation test with inhaled AMP was undertaken in a similar manner to that described for methacholine. FEV_1 measurements were recorded at 1 and 3 minutes after inhalation of each concentration of AMP, and the corresponding PC_{20} FEV_1 values were derived. On the

TABLE II. Effects of inhaled bumetanide, furosemide, and placebo on baseline FEV_1 values (in liters)

Subject No.	Baseline	Placebo	Bumetanide	Furosemide
1	3.30	3.20	3.25	3.20
2	2.30	2.35	2.35	2.35
3	3.50	3.60	3.50	3.75
4	2.60	2.70	2.90	2.85
5	2.35	2.30	2.25	2.30
6	3.10	2.90	3.20	3.15
7	2.10	2.10	2.00	2.20
8	2.30	2.50	2.25	2.20
9	4.10	4.05	4.20	4.30
10	2.40	2.40	2.50	2.30
11	3.10	3.05	3.05	3.15
12	3.50	3.35	3.55	3.50
Mean ± SEM	2.89 ± 0.18	2.88 ± 0.17	2.92 ± 0.19	2.94 ± 0.20

next three visits, subjects attended the laboratory to undertake concentration-response studies with inhaled AMP after nebulized furosemide, bumetanide, or matched nebulized vehicle (placebo) administered in double-blind fashion and in random order 10 minutes before challenge. On each occasion, after a 15-minute rest, three baseline measurements of FEV_1 were recorded at intervals of 3 minutes. This was followed by inhalation of nebulized furosemide (Lasix, Hoechst, Frankfurt AM Main, Germany) in a concentration of 10 mg/ml, nebulized bumetanide (Burinex, Leo, Aylesbury, England) in a concentration of 0.50 mg/ml, or nebulized vehicle consisting of 0.9% sodium chloride adjusted to similar pH and tonicity as the drug solution used. The aerosol solutions were generated from a starting volume of 4.0 ml in an Inspiron mini-nebulizer driven by compressed air at a rate of 6 L/min and inhaled to dryness by deep tidal breathing over 7- to 9-minute period. The same nebulizer was used for all studies on all subjects. The amount of furosemide and bumetanide delivered to the mouth was calculated by differential weighing and on the five occasions amounted to 26 ± 2.1 mg (mean ± SEM) and 1.4 ± 0.2 mg, respectively. Ten minutes after inhalation of the furosemide, bumetanide, or matched vehicle placebo, a concentration-response study with AMP was performed. On each occasion, three measurements of FEV_1 after drug administration were recorded at 3 and 5 minutes, followed by inhalation of nebulized 0.9% sodium chloride and repeat measurements of FEV_1 at 1 and 3 minutes. Provided that the FEV_1 did not fall by more than 10% of the baseline value after drug administration, the concentration-response study was undertaken. After each agonist concentration was inhaled, FEV_1 was measured at 1 and 3 minutes, and the higher value was recorded. Increasing doubling concentrations of AMP were then inhaled at 5-minute intervals until FEV_1 had fallen by more than 20% of the value after saline challenge or until the highest concentration had been administered.

In the second phase of the study, eight subjects (nos. 13 to 20) attended the laboratory on nine separate occasions, 3 to 7 days apart, in order to investigate the time course of changes in AMP bronchial reactivity with inhaled furosemide (40 mg) and bumetanide (2 mg). On each occasion, subjects received nebulized furosemide, bumetanide, or matched placebo at 10, 30, and 120 minutes before bronchial provocation test with AMP in a randomized, double-blind fashion. Thus on the nine visits required to complete this phase of the study, each subject received: (1) placebo solution at 10, 30, and 120 minutes; (2) furosemide at 10, 30, and 120 minutes; (3) bumetanide at 10, 30, and 120 minutes. The administration of the aerosol solutions and the challenge procedure used were identical to those described in phase 1 of the study.

Data analysis

Results are expressed as means ± SEM unless otherwise stated and $p < 0.05$ was accepted as the minimum level of statistical significance. Pre- and posttreatment baseline values of FEV_1 before bronchial challenges were compared within each study day by means of Student's t test for paired data and between study days by two-way analysis of variance (ANOVA). The repeatability of the AMP challenge procedure was determined according to the method described by Altman and Bland[24] in which the difference is plotted against the mean of the logarithmically transformed PC_{20} values obtained on the placebo and open study days. The mean and standard deviation (SD) of the difference between these values were then derived and used to calculate the coefficient of repeatability between the results of the 2 study days.

The slopes of the AMP concentration-response curves were determined by least squares linear regression analysis and compared between post-placebo, post-furosemide, and post-bumetanide study days with Student's t test for paired data. AMP PC_{20} values were logarithmically transformed to normalize their distribu-

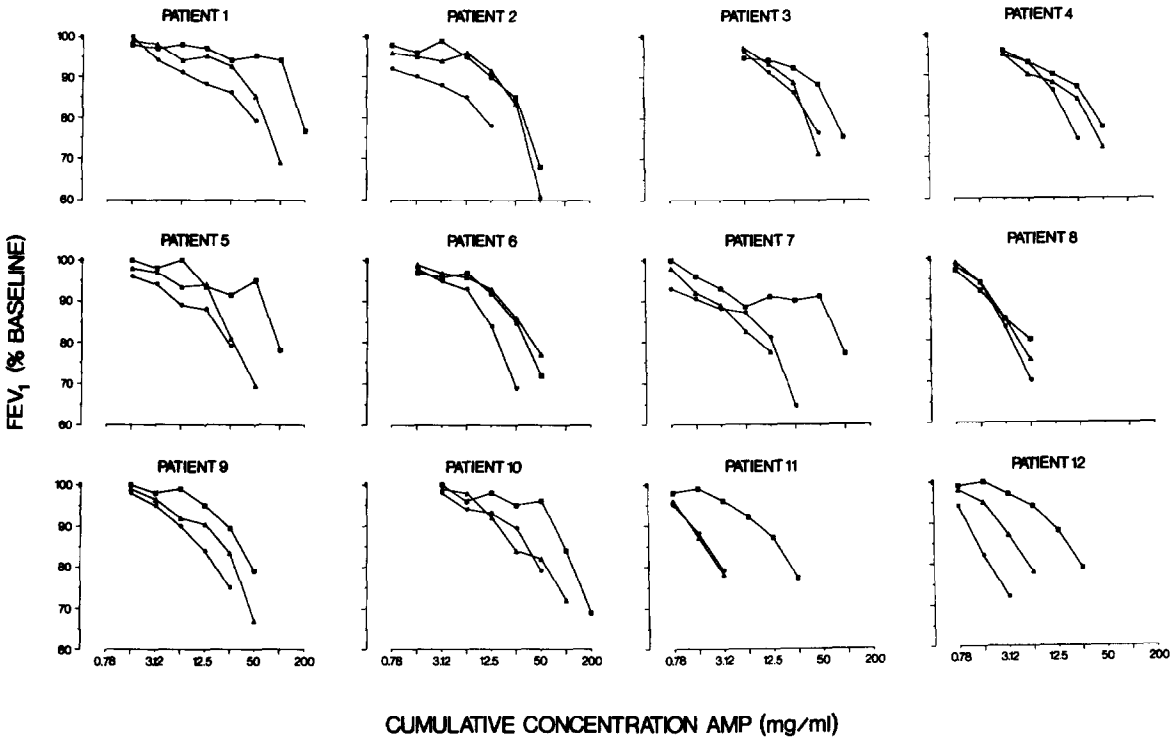

FIG. 1. Effect of inhaled placebo (●), furosemide (■), and bumetanide (▲) on the concentration-related falls in FEV_1 produced by inhaled AMP in 12 subjects with asthma.

TABLE III. Effects of inhaled bumetanide, furosemide, and placebo on airway AMP responsiveness

	PC_{20} AMP (mg/ml)		
Subject No.	**Placebo**	**Bumetanide**	**Furosemide**
1	96.88	120.91	344.95
2	12.54	48.06	53.91
3	55.44	49.71	148.29
4	30.34	58.06	76.25
5	41.82	46.75	178.33
6	25.16	78.54	62.21
7	25.28	21.88	169.12
8	5.70	7.17	11.33
9	23.89	48.16	83.96
10	88.79	108.40	221.00
11	5.12	4.69	33.69
12	2.47	9.50	40.19
Geometric mean	21.20	33.82	83.35
Range	2.5-96.9	4.7-120.9	11.3-345.0

tion and compared by means of two-factor ANOVA followed by Neuman-Keuls analysis as appropriate. However, because log transformation may be insufficient to normalize the data, we have also performed nonparametric statistical analysis of our data with Friedman two-way ANOVA. For each subject, concentration ratios for the protective effect of furosemide and bumetanide against bronchoprovocation with AMP were calculated individually by dividing the PC_{20} value obtained after administration of active drug by that obtained after administration of placebo. The relative efficacy of the two drugs in protecting the airways against bronchoconstriction provoked by AMP was derived for each subject by dividing the concentration ratio for furosemide by that for bumetanide and analyzed by means of Wilcoxon signed-rank test. Concen-

tration ratios for furosemide and bumetanide were correlated by least mean squares regression analysis on logarithmically transformed data.

RESULTS

Phase 1

There were no significant differences in mean baseline values of FEV_1 between any of the study days. Inhaled furosemide and bumetanide were well tolerated and had no effect on baseline FEV_1 (Table II). On the placebo study day the geometric mean (range) PC_{20} value with AMP was 21.2 mg/ml (2.5 to 96.9 mg/ml), which did not differ significantly from the value of 17.9 mg/ml (3.1 to 84.8 mg/ml) obtained on the open study day. The challenge procedure with AMP in this group of subjects was found to be repeatable with a coefficient of repeatability of 1.2 doubling dilutions and, for 10 of the 12 subjects, was within a single doubling dilution. These findings were consistent with the repeatability data obtained in previous studies with the AMP challenge.[6, 18-20]

When compared with placebo, both furosemide and bumetanide protected the airways against the constrictor effects of inhaled AMP. Furosemide produced a displacement of the AMP concentration-response curve to the right in all 12 subjects, whereas protection with bumetanide was observed only in nine subjects (Fig. 1) (Table III). Covariant analysis demonstrated that the slopes of the AMP concentration-response curves after administration of inhaled placebo and the two active drugs did not depart significantly from parallel. Inhaled furosemide had a significant protective effect against the fall in FEV_1 provoked by AMP (Fig. 2), the geometric mean (range) PC_{20} value increasing from 21.2 after placebo administration to 83.4 mg/ml (11.3 to 345.0 mg/ml) after administration of the drug ($p < 0.001$). Comparable equidiuretic doses of inhaled bumetanide were less effective in protecting against the AMP–provoked fall in FEV_1 (Fig. 2); the geometric mean PC_{20} value increased to 33.8 mg/ml (4.7 to 120.9 mg/ml) ($p < 0.05$). When expressed as concentration ratios, furosemide afforded a 3.9-fold (2.0 to 16.3-fold) protection of the airways against AMP, whereas that for bumetanide was 1.6-fold (0.9 to 3.9-fold). Thus in molar terms, furosemide was approximately 2.5 times more potent than bumetanide in attenuating the fall in FEV_1 provoked by AMP ($p < 0.01$). No correlation could be found between the degree of protection afforded by the two drugs ($r = 0.22$, $p = 0.49$).

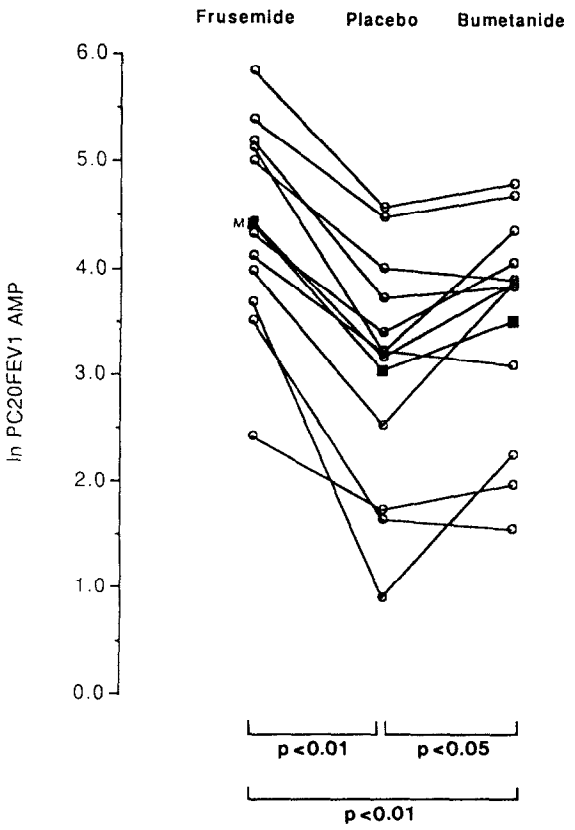

FIG. 2. Changes in provocation concentrations of AMP required to provoke a 20% decrease in FEV_1 (PC_{20}) after administration of placebo, furosemide, and bumetanide in 12 subjects with asthma. Solid squares *(M)* refer to geometric mean values.

Phase 2

There were no significant differences in mean baseline values of FEV_1 between any of the 9 study days. Neither furosemide nor bumetanide produced alteration in baseline FEV_1 values at any of the time points studied. When compared with placebo, both inhaled furosemide and bumetanide significantly reduced the airway responsiveness to AMP at 10 and 30 minutes, with a peak effect at the 10-minute time point for both drugs. After placebo administration, the PC_{20} AMP values at 10, 30, and 120 minutes did not differ significantly from each other; their geometric mean (range) values were 57.8 mg/ml (10.9 to 341.0 mg/ml), 55.0 mg/ml (13.2 to 304.1 mg/ml), and 52.8 mg/ml (14.4 to 252.2 mg/ml), respectively (Table IV). Pretreatment with furosemide increased the PC_{20} AMP values up to 154.6 mg/ml (29.4 to 658.7 mg/ml) ($p < 0.01$) and 142.6 mg/ml (25.5 to 639.9 mg/ml) ($p < 0.01$) at 10 and 30 minutes, respectively. Similarly, the PC_{20} values

TABLE IV. Individual $PC_{20}AMP$ values after inhaled bumetanide, furosemide, and placebo at different time points

Subject No.	$PC_{20}AMP$ (mg/ml)								
	B (10 min)	F (10 min)	P (10 min)	B (30 min)	F (30 min)	P (30 min)	B (120 min)	F (120 min)	P (120 min)
13	25.9	55.9	11.5	22.1	60.8	19.3	25.4	41.1	21.0
14	37.3	32.8	29.9	21.6	30.0	21.3	23.0	22.3	24.2
15	141.8	621.3	85.9	124.1	588.2	95.9	136.9	400.7	57.8
16	48.4	55.4	22.5	62.1	43.2	15.5	22.6	26.1	17.7
17	403.2	533.2	341.0	353.9	639.9	274.8	233.3	605.5	205.6
18	49.4	29.4	10.9	32.0	25.5	13.2	40.9	12.5	14.4
19	258.0	658.7	172.5	230.9	599.7	123.9	125.5	389.4	155.0
20	639.0	501.0	290.2	531.7	375.6	304.1	318.0	480.0	252.2
Geometric mean	110.2	154.6	57.8	92.0	142.6	55.0	71.5	103.9	52.8
Range	25.9-639.0	29.4-658.7	10.9-341.0	21.6-531.7	25.5-639.9	13.2-304.1	22.6-318.0	12.5-605.5	14.4-252.2

B, Bumetanide; *F*, furosemide; *P*, placebo.

for AMP 10 and 30 minutes after bumetanide administration were significantly increased up to 110.2 mg/ml (25.9 to 639.0 mg/ml) ($p < 0.01$) and 92.0 mg/ml (21.6 to 531.7 mg/ml) ($p < 0.05$), respectively (Table IV). However, although furosemide was still significantly effective in attenuating the airway response to AMP at 120 minutes compared with placebo, bumetanide failed to protect against AMP-induced bronchoconstriction at the same time point; the PC_{20} values for AMP were 52.8 mg/ml (14.4 to 252.2 mg/ml), 103.9 mg/ml (12.5 to 605.5 mg/ml), and 71.5 mg/ml (22.6 to 318.0 mg/ml) for placebo, furosemide, and bumetanide, respectively (Table IV).

DISCUSSION

The present study confirms the previous observation by us and others[6, 25] that inhaled furosemide protects the airways of subjects with asthma against bronchoconstriction provoked by inhaled AMP. The protection afforded by furosemide occurred in all of the subjects studied and amounted to approximately fourfold displacement of the concentration-response curves to the right, which is similar to that reported previously.[6, 25] Although comparable equidiuretic doses of bumetanide were also effective in inhibiting the airway effect of AMP, this drug was approximately 2.5 times less efficacious than furosemide. In addition, we have demonstrated that the time course of change in bronchial reactivity to AMP is quite similar, with a peak effect at 10 minutes, for both inhaled furosemide and bumetanide, thus indicating the validity of comparing relative bronchoprotective potencies of inhaled furosemide and bumetanide.

The mechanism of the protective activity of "loop" diuretics in asthma remains to be established. In providing protection against a variety of different bronchoconstrictor stimuli such as allergen,[1] fog,[2] exercise,[3] cold air,[4] sodium metabisulphate,[5] AMP,[6] and methacholine,[6] the inhibitory effects of "loop" diuretics may involve mechanisms common to all of these stimuli.

Drugs such as furosemide produce some of their effect in the kidneys by the secondary production of endogenous prostanoids.[26, 27] In human beings furosemide causes an increase in the plasma concentration of free arachidonic acid[28] and enhances the urinary excretion of prostaglandins including prostacyclin (PGI_2).[29] In rats, intravenously administered furosemide releases prostanoids that inhibit constrictor responses in the peripheral vasculature.[30] Taken together these data provide convincing evidence for furosemide's capacity to generate eicosanoids with functional effects. That this also occurs in the airways is supported by a recent report in which it has been demonstrated that bovine tracheal mucosa produces prostaglandin E_2 (PGE_2) in response to furosemide.[31] In addition, Pavord et al.[32] have recently shown that the protective effect of furosemide against exercise-induced asthma was abolished by cyclooxygenase blockade with indomethacin. It is suggested that furosemide affords protection against exercise and related stimuli by releasing PGE_2 and PGI_2, which are both potent functional antagonists because of their capacity to

stimulate adenylate cyclase in the airways.[33, 34] Both human airway tissue[35] and pulmonary vascular endothelial cells[36] are rich sources of PGI_2 and PGE_2. In asthma inhaled PGI_2 affords short-term protection against several stimuli such as exercise,[37] nebulized distilled water (fog),[37] PGD_2,[34] and methacholine,[34] without having any consistent effect on basal airway calibre. Similarly, inhaled PGE_2 protects against the contractile effect of sodium metabisulfite and methacholine in patients with asthma.[38] Release of PGE_2 and/or PGI_2 in response to inhaled "loop" diuretics could account for the protection against the airways' effect of AMP without changing basal airway calibre.

The capacity of inhaled furosemide and bumetanide to reduce the airways' response to exogenously administered bronchoprovocants may also result from a direct effect of these drugs on the bronchial epithelium and underlying vasculature. Through their effect on the Na-K-Cl cotransporter mechanism, "loop" diuretics may increase the chloride and water content of the periciliary fluid.[7] In addition, furosemide is an effective vasodilator[39] and, as a consequence, could enhance bronchial blood flow to increase the transepithelial clearance of an inhaled agonist such as AMP. That this also occurs in allergic airways is supported by a recent study in which it has been demonstrated that in sheep sensitized to *Ascaris suum* the magnitude and duration of the antigen-induced airway smooth muscle constriction were influenced by microvascular hyperfusion.[40]

Inhaled furosemide seems to exert a preferential protective effect against those stimuli that cause bronchoconstriction in part by releasing spasmogenic mediators from mast cells including allergen,[1] fog,[2] exercise,[3] and AMP.[6] Recently, Temple et al.[41] have shown that furosemide (10^{-6} to 10^{-3} mol/L) is capable of inhibiting the IgE-dependent release of histamine and leukotriene C_4 from human lung fragments. Thus a modulatory function on mast cells may be responsible for the protective effect of this and other "loop" diuretics on a variety of mast cell–dependent bronchoconstrictor stimuli. However, the demonstration of the protective effect of inhaled furosemide against methacholine[6] and sodium metabisulfite–induced bronchoconstriction[5, 25] suggests that inhibition of mast cells cannot be the sole mechanism of action of furosemide in asthma.

There is now some evidence that drugs such as furosemide might alter neural activity in the airways. Results of a recent study by Ventresca et al.[42] which showed that in healthy subjects inhaled furosemide inhibits cough responses induced by low-chloride aerosols, support an effect on sensory nerves. The bronchoconstrictor effect of AMP is significantly inhibited by cromones[43] and is attenuated to some extent by anticholinergic agents,[19] which suggests that excitation of neural pathways may be of some importance in this response. Our observation of a similar degree of protection against AMP challenge suggests that loop diuretics may act on these sensory pathways. Further evidence for an effect on nerves is supported by a recent investigation with guinea pig airways, showing that both furosemide and bumetanide inhibit the airway smooth muscle contraction induced by stimulation of cholinergic and noncholinergic nonadrenergic nerves in a dose-dependent manner and that this effect is independent of cyclooxygenase production.[44]

The finding that furosemide is appreciably more potent than equidiuretic doses of bumetanide in protecting against the airways' response to AMP and the lack of correlation between the degree of protection afforded by the two drugs suggest a mechanism that is independent of the Na-K-Cl cotransporter mechanism. These findings are in agreement with results of a recently reported randomized double-blind study, which demonstrated that inhaled furosemide and bumetanide effectively prevented "fog"-induced bronchoconstriction in subjects with asthma, with furosemide being significantly more potent than bumetanide in this respect.[45] Although both drugs share similar properties, it is not clear how to explain the observed difference in efficacy. Low concentrations of furosemide and bumetanide have different effects on prostanoid production and acute renin release. In vitro furosemide, but not bumetanide, has been shown to inhibit 15-hydroxyprostaglandin dehydrogenase, an enzyme involved in the inactivation of PGs.[46, 47] The difference in activity of the two drugs on prostanoid production and catabolism has functional consequences in that bumetanide and furosemide exhibit different effects on perfused rat and rabbit kidneys.[48] In human beings only low doses of intravenously administered furosemide, but not bumetanide, stimulate an acute elevation in plasma renin, which is paralleled by an increase in peripheral blood flow.[49] If the same applies to the microcirculation of the human bronchial mucosa, as recently indicated,[40] this could lead to an increased washout of the exogenously administered bronchoprovocants and could also account for

some of the difference in protective efficacy against AMP-induced bronchoconstriction that is observed with the two drugs.

In the present study we have extended our previous observations on the bronchoprotective properties of inhaled loop diuretics by investigating the duration of the change in AMP airway responsiveness with furosemide and bumetanide in a separate group of subjects with asthma. Both inhaled furosemide and bumetanide caused attenuation in bronchial reactivity to AMP, with the maximum changes occurring 10 minutes after drug administration and with a progressive reduction over the 2 hours of observation time. Although the maximum protective effect against AMP-induced bronchoconstriction was reported at the same time point for both drugs, furosemide was significantly more potent and had a longer duration of action compared with bumetanide. A possible reason for these findings is that although both furosemide and bumetanide are weak acids with an identical Pk_a of 3.5, bumetanide, in contrast to furosemide, is highly lipid-soluble[50] and may not have remained in efficacious doses at its site of action at the time of the bronchoprovocation tests.

In conclusion, we have further shown that AMP-induced bronchoconstriction is inhibited by inhaled furosemide, a property also shared to a lesser degree by bumetanide. The time course of this phenomenon is quite similar for both drugs, with a maximum protective effect at 10 minutes. Possible mechanisms of action include the secondary production of protective prostanoids, an increase in the water content of the airway lining fluid with enhanced AMP clearance, a direct inhibitory effect on mast cell activation-secretion coupling or a modulation of the nervous activity of the airways. The differences in the protective effect afforded by equidiuretic doses of furosemide and bumetanide may reflect a mechanism of action distinct from that responsible for their diuretic properties. Further research is needed to clarify the intriguing suppressive action of this class of drugs on constrictor stimuli in asthma and to determine whether this has any relevance to a possible therapeutic effect in this disease.

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