

Respiratory pathophysiologic responses

Effect of acetazolamide on cough induced by low-chloride-ion solutions in normal subjects: Comparison with furosemide

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Background: The antitussive activity of inhaled furosemide has been attributed to its blocking effect on the $\text{Na}^+-2\text{Cl}^--\text{K}^+$ cotransporter. It is likely that the antitussive activity of inhaled diuretics is more complex because amiloride, a diuretic that has no effect on the $\text{Na}^+-2\text{Cl}^--\text{K}^+$ cotransporter, also shows a significant effect against cough induced by low-chloride-ion solutions. Apart from pharmacokinetics of inhaled diuretics, this activity could also depend on the inhibition of carbonic anhydrase.

Objectives: We therefore studied the effect of inhaled acetazolamide, a selective inhibitor of carbonic anhydrase activity, on cough induced by the inhalation of different chloride ion solutions in a group of normal subjects. This was compared with the antitussive effect of furosemide. In addition, we attempted to determine whether the effect of acetazolamide is dose-dependent.

Methods: Cough challenge consisted of consecutive inhalations of four solutions having decreasing concentrations of chloride ions (150, 75, 37.5 and 0 mmol/L). Nine normal subjects underwent the cough challenge 5 minutes after the inhalation of saline placebo, acetazolamide (500 mg), and furosemide (30 mg) according to a randomized, double-blind study design. A group of six subjects were challenged according to the same procedure and study design, after the inhalation of saline placebo and of two doses of acetazolamide (250 mg and 500 mg).

Results: Inhaled acetazolamide significantly reduced cough response to 37.5 and 0 mmol/L chloride solutions compared with placebo ($p < 0.015$ and $p < 0.015$, respectively). Furosemide showed a similar protective effect ($p < 0.015$ and $p < 0.025$, respectively). Acetazolamide provided a significantly better protective effect than furosemide ($p < 0.025$ and $p < 0.015$, respectively). The antitussive activity of the two doses of acetazolamide was not statistically different.

Conclusion: These results demonstrate that inhaled acetazolamide, a selective inhibitor of carbonic anhydrase, attenuates cough induced by low-chloride-ion solutions in normal subjects. At the applied doses, its antitussive activity is slightly greater than furosemide. This finding suggests that the inhibition of carbonic anhydrase activity is likely involved in modulating changes caused by absence of a chloride ion in the airway microenvironment of human beings. (*J Allergy Clin Immunol* 1996;97:1093-9.)

Key words: Furosemide, acetazolamide, cough challenge

Inhaled furosemide exhibits a variable degree of protection against bronchoconstriction induced by several stimuli in asthmatic subjects.^{1, 2} In addition, inhaled furosemide has been shown to significantly

reduce cough induced by the inhalation of low-chloride-ion solutions³ and prostaglandin $\text{F}_{2\alpha}$ ⁴ in normal individuals, whereas cough induced by citric acid⁵ and capsaicin³ is not modified. It is

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conceivable that furosemide could modulate induced cough by controlling neural discharge by sensory afferent nerve endings from the airways. An effect of inhaled furosemide on airway nerve endings is supported by its protective effect against smooth-muscle contraction induced by cholinergic stimulation in *in vitro* preparations.⁶ Furthermore, Sant'Ambrogio et al.⁷ have recently demonstrated that cough induced by low-chloride solutions stimulates laryngeal irritant receptors of dogs, and these receptors are blocked by the administration of furosemide.

The antitussive effect of furosemide has been attributed to an inhibition of the $\text{Na}^+2\text{Cl}^- \text{K}^+$ cotransporter at the level of neuronal tissue.¹ However, the mechanism is probably more complex because amiloride, a diuretic that has no effect on the $\text{Na}^+2\text{Cl}^- \text{K}^+$ cotransporter, also has a small but significant effect against cough induced by low-chloride-ion solutions.⁸ Moreover, furosemide likely has an acetazolamide-like effect, as suggested by its affinity for carbonic anhydrase in the choroid plexus.⁹

Therefore to test the hypothesis that the antitussive effect of inhaled diuretics is dependent on inhibition of carbonic anhydrase activity, we compared the antitussive effect of inhaled acetazolamide, a potent inhibitor of carbonic anhydrase activity,¹⁰ with that of furosemide. We examined the cough response to low-chloride-ion solutions in normal subjects before and after treatment with inhaled acetazolamide and furosemide and attempted to determine whether the effect of acetazolamide was dose-dependent.

METHODS

Subjects

A group of 13 subjects (10 women; mean age, 24 years; range, 19 to 38 years) of 23 normal subjects were enrolled in the study because they showed a positive cough response to the inhalation of low-chloride-ion solutions. Only subjects demonstrating a cough response greater than 8 coughs were selected.¹¹ None had personal or family history of atopic diseases. All subjects had been free of upper and lower respiratory tract infections for at least 6 weeks before the study. None of them showed bronchial responsiveness to methacholine ($\text{PD}_{20} \text{FEV}_1 > 23 \mu\text{mol}$).¹² None had a history of spontaneous coughing. All subjects gave informed consent to participate in the study, and the protocol was approved by the hospital ethics committee.

Study design

Subjects attended the laboratory at the same time of day between 9:00 and 11:00 AM on each occasion. In the

first part of the study, a total of five cough challenges were performed in each subject within 14 days. Cough challenge without any premedication was performed in each subject before entry into the study and after the study was completed. The effect of inhaled furosemide and acetazolamide on cough challenge was examined in a double-blind, randomized, placebo-controlled fashion. Each subject received acetazolamide (500 mg), furosemide (30 mg), or placebo (saline solution) on 3 separate days, at least 2 days apart. On each day of the study, the cough challenge was performed 5 minutes after the inhalation of drugs and placebo. FEV_1 was measured in duplicate by a computerized spirometer (Altair 1000; Cosmed S. R. L., Rome, Italy) before and after treatments and at the end of the cough challenge. A group of nine subjects completed the protocol. In the second part of the study, the effect of two different doses of inhaled acetazolamide, namely 250 and 500 mg, on cough challenge was examined according to a double-blind, randomized, placebo-controlled design. Active drugs and placebo were administered on separate days, at least 2 days apart. On each day of the study, the cough challenge was performed 5 minutes after the inhalation of drugs and placebo. A group of six subjects completed this part of the study. Only two subjects participated in both parts of the study. Treatments were inhaled through a mouthpiece during tidal breathing.

Cough challenge

The cough challenge consisted of consecutive inhalations of four iso-osmolar solutions with decreasing chloride ion concentrations (150, 75, 37.5, and 0 mmol/L), as previously described.¹¹ The pH values of the four solutions were 6.36, 8.18, 8.19, and 8.22, respectively. The osmolarity values were 290.5, 277.5, 271.5, and 264.5 mmol/L, respectively. Solutions were freshly made every day with 1.26% sodium bicarbonate and 0.9% saline. Each solution was inhaled for 1 minute at 3-minute intervals through an ultrasonic nebulizer (DeVilbiss 65, mean output 2.0 ml/min; De Vilbiss Medizinische GmbH, Langen, Germany). Each subject inhaled the solutions through a mouthpiece, and the number of coughs was counted during the 1-minute inhalation by an observer who was not aware of the study protocol. The cough response was expressed as the number of coughs per minute.

Drugs

Acetazolamide, 500 mg of dry powder, was reconstituted in 12 ml of sterile water (pH = 8.62; osmolarity = 892 mmol/L) and further diluted to obtain a 250 mg dose in 12 ml (pH = 8.04; osmolarity = 490 mmol/L). Furosemide (stock solution, 10 mg/ml) was diluted with 0.9% saline solution to obtain 30 mg of furosemide in a total volume of 12 ml (pH = 8.75; osmolarity = 209 mmol/L). Placebo solution consisted of 12 ml of 0.9% saline solution (pH = 8.4; osmolarity = 441 mmol/L). All solutions were nebulized to dryness by a continuous aerosol (Artsana SP1000 [Artsana Spa, Como, Italy]; flow = 22 L/min; driving pressure = 283 kPa; mean

TABLE I. Characteristics of study population

Subject No.	Age (yr)	Weight (kg)	Height (cm)	Smoke	Baseline FEV ₁ (L)	PD20M (μmol)
1	29	52	168	N	3.24	>23
2	23	70	160	Ex	4.08	>23
3	38	80	188	Ex	4.13	>23
4	24	73	187	N	5.20	>23
5	24	46	156	N	2.89	>23
6	25	43	163	N	2.69	>23
7	20	52	140	N	2.41	>23
8	24	54	157	N	2.51	>23
9	20	65	162	Ex	3.41	>23
10	23	60	155	N	3.30	>23
11	19	69	168	N	3.30	>23
12	21	67	156	N	3.10	>23
13	21	54	170	N	3.10	>23

Subjects 1 to 9 participated in the first part of the study; subjects 8 to 13 participated in the second part of the study. *N*, Nonsmoker; *Ex*, ex-smoker; *PD20M*, provocative dose of methacholine.

output = 1.4 ml/min, by multiple weighing) over a period of approximately 10 minutes.

Statistical analysis

The analysis was restricted to cough response measured after the inhalation of 37.5 and 0 mmol/L solutions, because very few subjects coughed after inhaling solutions with higher chloride concentrations. Results are given as means and standard errors. The reproducibility of cough response induced by the low-chloride-ion solutions was assessed by the intraclass correlation coefficient. Friedman's analysis was used to compare changes in cough response after treatments, and differences between placebo and each active treatment were assessed by using Wilcoxon's rank sum analysis with Bonferroni's correction. Percent changes in baseline FEV₁ values induced by treatments and low-chloride-ion solutions were examined by analysis of variance. A statistical significance was always taken at $p < 0.05$.

RESULTS

Characteristics of the nine subjects who participated in the first part of the study are shown in Table I. The baseline cough response after inhaling 37.5 mmol/L and 0 mmol/L chloride solutions assessed before and after the double-blind study was reproducible (intraclass correlation coefficients: 0.85 and 0.87, respectively).

There were no significant differences in baseline FEV₁, expressed as percent of predicted values. Inhaled diuretics did not significantly change FEV₁ values. Mean baseline FEV₁ ($\pm$ SD) was 100.1% $\pm$ 3.2% in the placebo group; 99.1% $\pm$ 3.1% in the acetazolamide group; and 98.2% $\pm$ 2.4% in the furosemide group. Mean percent

changes in the three groups were 1.3% (placebo group), 1.8% (acetazolamide group), and 2.5% (furosemide group). Moreover, FEV₁ values measured after the cough challenge were not different from baseline (97.4% $\pm$ 4.1%; 100.3% $\pm$ 2.7%, and 98.7% $\pm$ 3.3%, respectively).

Number of coughs per minute recorded in each subject after administration of placebo or diuretics is reported in Table II, and mean values ($\pm$ SEM) are shown in Fig. 1. After acetazolamide administration, cough response to 37.5 mmol/L and 0 mmol/L chloride solutions was significantly reduced as compared with placebo ($p < 0.015$ and $p < 0.015$, respectively). A similar trend was also evident after administration of furosemide ($p < 0.015$ and $p < 0.025$, respectively). Acetazolamide provided a significantly better protective effect than furosemide ($p < 0.025$ and $p < 0.015$, respectively).

Characteristics of the six subjects who participated in the second part of the study are shown in Table I. Number of coughs per minute recorded in each subject after administration of placebo and the two doses of acetazolamide are reported in Table III, and mean values ($\pm$ SEM) are shown in Fig. 2. After inhalation of 250 mg of acetazolamide, cough response to 37.5 mmol/L and 0 mmol/L chloride solutions was significantly reduced as compared with cough response to placebo ($p < 0.03$ and $p < 0.03$, respectively). A similar reduction was measured after the inhalation of 500 mg of acetazolamide ($p < 0.03$ and $p < 0.03$, respectively). There were no statistically significant differences between the two doses.

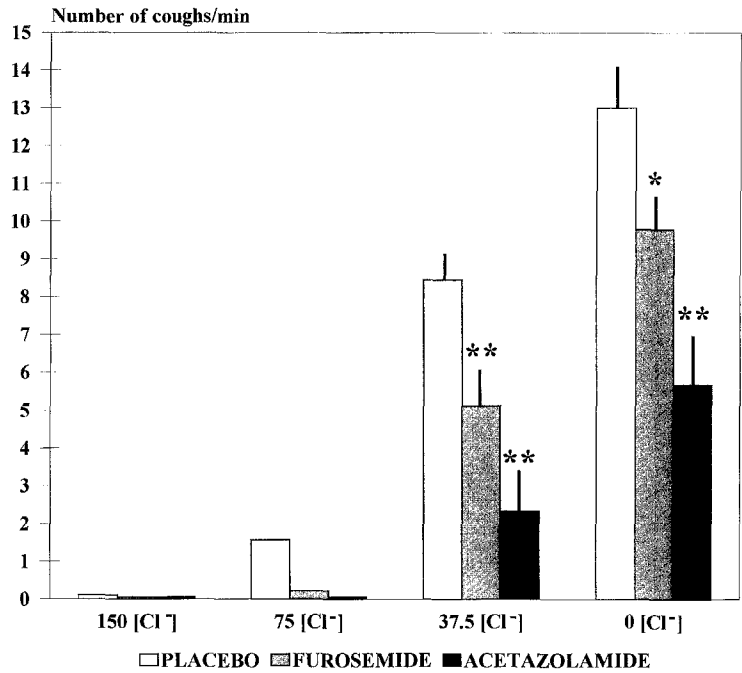


FIG. 1. Effect of placebo, 30 mg of furosemide, and 500 mg of acetazolamide on cough induced by low-chloride-ion solutions in nine normal subjects. Values are means $\pm$ SEM. * $p < 0.025$; ** $p < 0.015$ compared with placebo.

TABLE II. Cough response to 37.5 and 0 mmol/L chloride ion solutions on the different days of the study

Chloride concentration (mmol/L)	Control 1		Placebo		Acetazolamide		Furosemide		Control 2	
	37.5	0	37.5	0	37.5	0	37.5	0	37.5	0
1	10	11	10	15	7	9	10	10	8	11
2	9	19	8	14	0	3	1	13	10	16
3	10	10	13	11	2	5	6	8	4	11
4	8	15	6	10	1	2	5	11	6	12
5	2	12	7	18	0	12	6	11	3	12
6	7	12	6	10	0	5	3	10	3	13
7	5	13	9	12	0	1	5	5	6	13
8	10	19	11	17	11	14	9	16	13	16
9	2	11	6	10	0	0	1	4	0	12
Mean $\pm$ SEM	7.0 $\pm$ 1.1	13.6 $\pm$ 1.1	8.4 $\pm$ 0.8	13.0 $\pm$ 1.0	2.3* $\pm$ 1.3	5.6* $\pm$ 1.6	5.1† $\pm$ 1.0	9.8* $\pm$ 1.2	5.9 $\pm$ 1.3	12.9 $\pm$ 0.6

* $p < 0.015$ by comparison with placebo.

† $p < 0.025$ by comparison with placebo.

DISCUSSION

In this study we demonstrated that inhaled acetazolamide provides significant protection against cough induced by low-chloride-ion solutions in normal subjects. At the applied doses, its antitussive activity is greater than that produced by furosemide. By administering 250 mg and 500 mg of acetazolamide, no dose-dependent effect against induced cough was evident.

Cough induced by low-chloride-ion solutions is likely mediated by ion changes of the epithelial cells, which indirectly stimulate myelinated afferent nerve endings within the airways, or is produced by their direct activation. The triggering event of cough is the absence of the permanent ion, chloride, in the inhaled solution,¹³ which stimulates rapidly adapting receptors.⁷ Rapidly adapting receptors are present in the larynx and in the

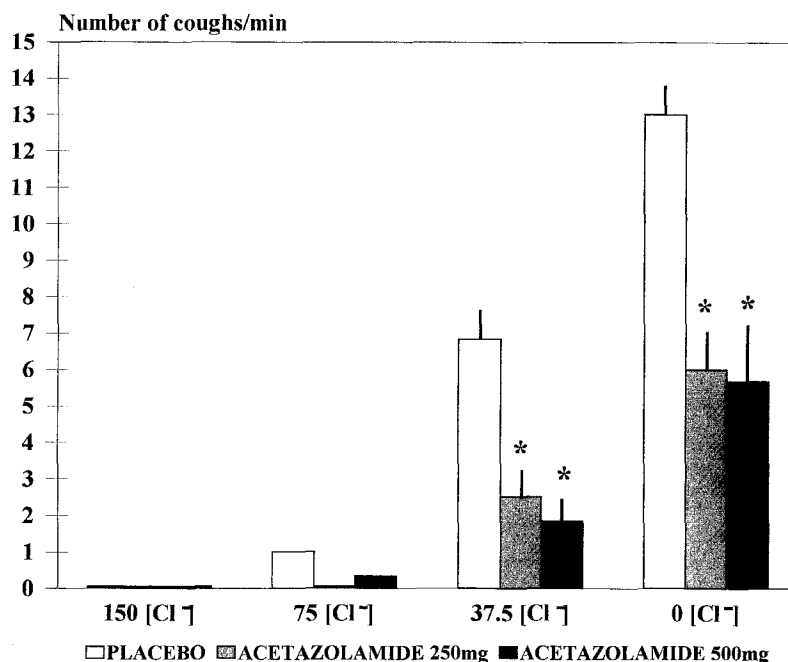


FIG. 2. Effect of placebo and acetazolamide (250 mg and 500 mg) on cough induced by low-chloride-ion solutions in six normal subjects. Values are means $\pm$ SEM. * $p < 0.03$ compared with placebo.

TABLE III. Effect of two doses of inhaled acetazolamide on cough response to 37.5 and 0 mmol/L chloride ion solutions

Chloride concentration (mmol/L)	Control		Placebo		ACTZ 250 mg		ACTZ 500 mg	
	37.5	0	37.5	0	37.5	0	37.5	0
1	7	13	8	11	3	7	4	6
2	12	23	6	15	2	5	2	8
3	7	10	6	15	4	10	3	10
4	7	10	3	10	0	3	0	0
5	15	14	7	12	6	11	2	9
6	10	12	11	15	0	0	0	1
Mean $\pm$ SEM	9.7 $\pm$ 1.4	13.7 $\pm$ 1.9	6.8 $\pm$ 1.0	13.0 $\pm$ 0.9	2.5* $\pm$ 0.9	6.0* $\pm$ 1.7	1.8* $\pm$ 0.6	5.7* $\pm$ 1.7

ACTZ, Acetazolamide.

* $p < 0.03$ by comparison with placebo.

tracheobronchial airways¹⁴ and are superficially located and sensitive to changes in chloride content in the surrounding microenvironment.^{15, 16} Under physiologic conditions, both neuronal cells and epithelial cells are able to modulate ion gradients, which control resting membrane potentials and volume, by means of powerful ion transport mechanisms. They comprise the Na⁺-2Cl⁻-K⁺ cotransporter and the bicarbonate exchanger.^{17, 18} The latter mechanism is under the control of carbonic anhydrase activity. The Na⁺-2Cl⁻-K⁺ cotransporter has been demonstrated

to be present in the peripheral neurons of several species¹⁹⁻²¹ and in the epithelial cells of animals and human beings.¹⁸ Carbonic anhydrase is also present in the afferent nerve endings of different animal species²²⁻²⁴ and controls intracellular pH and cell volume.¹⁷ In addition, a bicarbonate exchanger has been found on the serosal side of epithelial cells of horses²⁵ and in cultured epithelial cells from human beings.²⁶ Acetazolamide is a selective inhibitor of carbonic anhydrase.¹⁰ Although in the lungs of animals and human beings this enzyme activity is

highly concentrated in the endothelium of pulmonary capillaries, it is also present in the afferent sensory nerve endings of different animal species.^{22, 27} The inhibition of carbonic anhydrase decreases their excitability through an intracellular increase in CO_2 tension and changes in pH. Therefore HCO_3^- efflux, stimulated by changes in the surrounding environment, is prevented. A bicarbonate exchange system has also been demonstrated in animal²⁵ and human epithelium.²⁶ It appears that the $\text{Cl}^-/\text{HCO}_3^-$ exchange sustains the baseline chloride secretion. Whether inhaled acetazolamide concentrates at the level of the airways is not known. However, acetazolamide has a low lipid solubility.² Interestingly, inhaled acetazolamide has a protective effect comparable to that of furosemide against bronchoconstriction induced by cold air²⁸ and attenuates metabisulfite²⁹ and distilled water-induced bronchoconstriction.³⁰ Furthermore, acetazolamide significantly reduces in vitro smooth-muscle contraction induced by electrical field stimulation in guinea pig bronchi.³¹ This effect was dose-dependent, and reduction was approximately equal to 40%. When compared with other applied diuretics, the inhibition of induced contraction seems to parallel the inhibitory activity on carbonic anhydrase.³¹ Although the inhibition of bronchoconstriction does not necessarily imply a similar effect on induced cough, it is tempting to speculate that the antitussive effect of acetazolamide is related to a partial inhibition of neural discharge from afferent nerve endings within the airways. On the other hand, the absence of any dose-dependent effect suggests that the maximal achievable antitussive effect was obtained even with the lower dose of acetazolamide. Alternatively, it is also possible that cough induced by low-chloride-ion solution is in part mediated by nonmyelinated C fibers.

The antitussive effect of furosemide has been related to its interaction with the $\text{Na}^+-2\text{Cl}^--\text{K}^+$ cotransporter at the site of airway epithelium. Although this carrier is present in the luminal side of an intact epithelium,³² furosemide is also active when applied to the serosal side.¹ This effect may be due to a diffusion of furosemide but also to its pharmacokinetics. Because the lipid solubility of furosemide at physiologic pH is rather low,² it likely remains at the site of action long enough to exert its action. This may explain why other loop diuretics with greater potency on the $\text{Na}^+-2\text{Cl}^--\text{K}^+$ carrier but higher lipid solubility have a

lower protective effect on induced bronchoconstriction.³³ Moreover, it also accounts for furosemide's prolonged effect on induced cough.³⁴ Although the concentration achieved by furosemide within the airways is not known, it is probably rather high. Therefore an effect on carbonic anhydrase activity cannot be ruled out.^{2, 9} An additional site for furosemide is the $\text{Na}^+-2\text{Cl}^--\text{K}^+$ cotransporter at the site of afferent nerve endings. A direct effect at this level is supported by the observation that in single fiber experiments it blocks in vivo activation of rapidly adapting receptors in the larynx of dogs⁷ and inhibits in vitro contraction of bronchial smooth muscle preparations of guinea pigs induced by electrical field stimulation.⁶

In conclusion, this study demonstrates that acetazolamide has an antitussive activity against cough induced by low-chloride-ion solutions slightly greater than that of furosemide in normal subjects. However, this effect is evident when the inhaled dose of acetazolamide is at least eight times higher than furosemide. Our results suggest that the inhibition of carbonic anhydrase activity is likely involved in modulating changes caused by the absence of the chloride ion in the airway microenvironment. Whether this effect takes place at the site of airway epithelium or myelinated afferent nerve endings needs to be established.

REFERENCES

1. Bianco S, Pieroni MG, Refini RM, Robuschi M, Vaghi A, Sestini P. Inhaled loop diuretics as potential new anti-asthmatic drugs. *Eur Respir J* 1993;6:130-4.
2. Foresi A, Pelucchi A, Mastropasqua B, Carlesi RM, Marazzini L. Clinical potential of inhaled diuretics. *Clin Immunother* 1994;1:339-405.
3. Ventresca PG, Nichol GM, Barnes PJ, Fan Chung K. Inhaled furosemide inhibits cough induced by low chloride content solutions but not by capsaicin. *Am Rev Respir Dis* 1990;142:143-6.
4. Stone RA, Yeo CT, Barnes PJ, Chung KF. Furosemide inhibits cough but not bronchoconstriction to prostaglandins $\text{F}_{2\alpha}$ in patients with asthma. *Am Rev Respir Dis* 1991;143:A548.
5. Robuschi M, Vaghi A, Gambaro G, et al. Effect of inhaled furosemide on cough response to citric acid in normal subjects. *Prog Respir Res* 1990;24:117-21.
6. Elwood W, Lotvall JO, Barnes PJ, Chung KF. Loop diuretics inhibit cholinergic and noncholinergic nerves in guinea pigs. *Am Rev Respir Dis* 1991;143:1340-4.
7. Sant'Ambrogio FB, Sant'Ambrogio G, Anderson JW. Effect of furosemide on the response of laryngeal receptors to low-chloride solutions. *Eur Respir J* 1993;6:1151-5.
8. Stone RA, Fuller RW, Barnes PJ. Amiloride reduces cough induced by low (Cl) solution but not capsaicin [Abstract]. *Thorax* 1994;281P.
9. Vogh BP, Langham MR. The effect of furosemide and

- bumetanide on cerebrospinal fluid formation. *Brain Res* 1981;221:171-83.
10. Preisig PA, Toto RD, Alpern RJ. Carbonic anhydrase inhibitors. *Renal Physiol* 1987;10:136-59.
11. Caviglioli G, Sale M, Pelucchi A, et al. Prevalence and repeatability of cough response in normal subjects after inhalation of chloride ion solutions [Abstract]. *Eur Respir J* 1993;6:595s.
12. Foresi A, Mattoli S, Corbo GM, Polidori G, Ciappi G. Comparison of bronchial responses to ultrasonically nebulized distilled water, exercise, and methacholine in asthma. *Chest* 1986;90:822-6.
13. Eschenbacher WL, Boushey HA, Sheppard D. Alteration in osmolarity of inhaled aerosols causes bronchoconstriction and cough, but absence of a permeant anion causes cough alone. *Am Rev Respir Dis* 1984;129:211-5.
14. Karlsson J-A, Sant'Ambrogio G, Widdicombe J. Afferent neural pathways in cough and reflex bronchoconstriction. *J Appl Physiol* 1988;65:1007-23.
15. Boushey HA, Richardson PS, Widdicombe JG, Wise JCM. The response of laryngeal afferent fibers to mechanical and chemical stimuli. *J Physiol* 1974;240:153-75.
16. Boggs DF, Bartlett D Jr. Chemical specificity of a laryngeal apneic reflex in puppies. *J Appl Physiol* 1982;53:455-62.
17. Schue WR, Deitmer JW. Ionic mechanisms of intracellular pH regulation in the nervous system. *Ciba Found Symp* 1988;139:47-69.
18. Welsh MJ. Electrolyte transport by airway epithelia. *Physiol Rev* 1987;67:1143-84.
19. Gallagher JP, Nakamura J, Gallegher PS. The effects of temperature, pH and Cl^- pump inhibitors on GABA responses recorded from cat root ganglia. *Brain Res* 1983;267:249-59.
20. Russel JM. Cation-coupled chloride influx in squid axon. *J Gen Physiol* 1983;81:908-25.
21. Ballanyi K, Grafe P. An intracellular analysis of γ -aminobutyric-acid-associated ion movements in rat sympathetic neurons. *J Physiol* 1985;365:41-58.
22. Riley DA, Sanger JR, Matloub HS, Yousif HJ, Bain JL, Moore GH. Identifying motor and sensory myelinated axons in rabbit peripheral nerves by histochemical staining for carbonic anhydrase and cholinesterase. *Brain Res* 1988;453:79-88.
23. Szabolcs MJ, Windisch A, Koller R, Pensch M. Axon typing muscle nerves using a double staining procedure for cholinesterase and carbonic anhydrase. *J Histochem Cytochem* 1991;39:1617-25.
24. Suzuki AG, Kameyama J, Tsukamoto M, Kaneko K, Suzuki Y. Stimulation of Cl^- and HCO_3^- secretion by intramural cholinergic neurons in guinea pig antrum in vitro. *Am J Physiol* 1993;264:G118-25.
25. Tessier GJ, Traynor TR, Kannan MS, O'Grady SM. Mechanisms of sodium and chloride transport across equine tracheal epithelium. *Am J Physiol* 1990;259:L459-67.
26. Smith JJ, Welsh MJ. cAMP stimulates bicarbonate secretion across normal, but not cystic fibrosis airway epithelia. *J Clin Invest* 1992;89:1148-53.
27. Droz B, Kazimierzczak J. Carbonic anhydrase in primary sensory neurons of dorsal root ganglia. *Comp Biochem Physiol* 1987;88:713-7.
28. O'Donnel WJ, Rosenberg MA, Niven RW, Drazen JM, Israel E. Inhaled acetazolamide attenuates bronchoconstriction by cold-air hyperventilation. *Am Rev Respir Dis* 1992;146:1518-23.
29. O'Connor BJ, Yeo CT, Chen-Worsdell MM, Barnes PJ, Chung KF. Sodium metabisulphite induced bronchoconstriction is attenuated by a carbonic anhydrase inhibitor [Abstract]. *Thorax* 1992;47:234P.
30. Vaghi A, Robuschi M, DeBernardi G, et al. Protective effect of inhaled acetazolamide against ultrasonically nebulized water-induced bronchoconstriction: a dose-response study. *Am J Respir Crit Care Med* 1995;151:A178.
31. Sun J, Elwood W, Barnes PJ. Effect of thiazide diuretics against neurally mediated contraction of guinea pig airways. Contribution of carbonic anhydrase. *Am Rev Respir Dis* 1993;148:902-8.
32. Welsh MJ. Mechanisms of airway epithelial ion transport. *Clin Chest Med* 1986;7:273-83.
33. Foresi A, Pelucchi A, Mastropasqua B, Caviglioli G, Carlesi RM, Marazzini L. Effect of inhaled furosemide and tolasemide on bronchial response to ultrasonically nebulized distilled water in asthmatic subjects. *Am Rev Respir Dis* 1992;146:364-8.
34. Stone RA, Barnes PJ, Chung KF. Effect of furosemide on cough responses to chloride-deficient solution in normal and mild asthmatic subjects. *Eur Respir J* 1993;6:862-7.

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