Differential effects of furosemide on porcine bronchial arterial and airway smooth muscle

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Corboz, Michel R., Stephen T. Ballard, Hong Gao, Joseph N. Benoit, Sarah K. Inglis, and Aubrey E. Taylor. Differential effects of furosemide on porcine bronchial arterial and airway smooth muscle. J Appl Physiol 89: 1360–1364, 2000.—Furosemide attenuates airway obstruction in asthmatic subjects when administered as an aerosol pretreatment. This protective effect of furosemide could be related to relaxation of bronchial smooth muscle or to increased bronchial blood flow. To determine whether furosemide dilates bronchial smooth muscle, isometric contractile responses in distal bronchi from young pigs were studied. In bronchial smooth muscle rings that were precontracted with $10^{-5}$ M acetylcholine, significant relaxation occurred with $10^{-8}$ to $3 \times 10^{-6}$ M isoproterenol but not with $10^{-3}$ to $10^{-3}$ M furosemide. In contrast, bronchial arteries that were precontracted with either $10^{-4}$ M norepinephrine or $10^{-3}$ M vasopressin significantly relaxed in response to $10^{-4}$ to $3 \times 10^{-3}$ M and $10^{-3}$ to $3 \times 10^{-3}$ M furosemide, respectively. We conclude that furosemide, under the described experimental conditions, relaxes airway vascular smooth muscle but not bronchial smooth muscle. These results are consistent with previous suggestions that inhaled furosemide increases blood flow to airway tissues (Gilbert IA, Lenner KA, Nelson JA, Wolin AD, and Fouke JM. J Appl Physiol 76: 409–415, 1994).

When inhaled as an aerosol, furosemide offers protection in asthmatic subjects against several indirect bronchoconstrictor stimuli, including exercise (3), ultrasonically nebulized distilled water (20), cold air hyperventilation (11), sodium metabisulfite (16), adenosine (17), and both the early and the late responses to allergens (2). Recently, Tanigaki and colleagues (25) reported that inhaled furosemide rapidly reversed obstruction in patients with severe, acute asthma who were unresponsive to conventional therapy. Because aerosol administration of this agent is expected to produce few side effects, furosemide has promising clinical application in the treatment of this potentially fatal disease.

The antiasthmatic mechanism of furosemide is not well understood, but it is clearly unrelated to the diuretic actions of this drug (11, 17). As the result of an intra-airway thermodynamic analysis of exercise-induced asthma, Gilbert et al. (9) suggested that the antiasthmatic property of furosemide is related to dilation of airway vasculature, which should increase blood flow to the tissue and reduce the thermal gradient for heat exchange across the airway wall. This notion is supported by observations that furosemide dilates rat tracheal arterioles and venules in vivo (5). Inhaled furosemide has no effect against bronchoconstriction induced by histamine (8, 17), methacholine (11), or PGF2α (24), indirectly suggesting that this agent does not dilate airway smooth muscle. However, in vitro studies produce varying results, with some suggesting that furosemide dilates airway smooth muscle (23) but others reporting that it does not (7, 12).

In the present study, we hypothesized that furosemide dilates airway vasculature but not airway smooth muscle. To test this theory, we isolated bronchial arteries and bronchial smooth muscle from domestic pigs, a species having lung anatomy and physiology closely that resemble that of humans, and measured the in vitro contractile responses of these tissues to furosemide.

MATERIALS AND METHODS

Airway Excision

Young pigs (10.0–16.5 kg) of both sexes were obtained from a local vendor. Animals were sedated with intramuscular injections of ketamine (8.0 mg/kg) and xylazine (0.4 mg/kg) and then euthanized with an intravenous overdose of pentobarbital sodium. The chest was rapidly opened, and a distal portion of the right or left lung was excised.

Bronchial Rings

Preparation. Distal bronchi (~2–4 mm OD, 4–9 mm in length) were dissected from the surrounding lung parenchyma. Bronchial rings were suspended in 10-ml organ baths containing warm (37°C) Krebs buffer that was gassed with 95% O2–5% CO2, and the rings were then attached to isometric force transducers (Radnoti Glass Technology). Transducer output was recorded on a physiograph (Grass Instruments, Inc., Quincy, MA).

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tissues received only the drug vehicle. This concentration of acetylcholine produces 20–25% of the maximum constrictor response in porcine bronchial smooth muscle (unpublished observations). This priming sequence was repeated (typically 1–2 times) until sequential tension responses were within 10% of one another. When the priming sequence was completed, 10^{-8} M acetylcholine was added, and either furosemide (from 10^{-8} to 10^{-3} M) or isoproterenol (from 10^{-8} to 3 \times 10^{-6} M), a nonselective \beta-adrenergic-receptor agonist, was added to the bath in sequential, increasing concentrations. The furosemide vehicle was saline at 10^{-8} to 3 \times 10^{-6} M and dimethylformamide at 10^{-7} to 10^{-5} M. The isoproterenol vehicle was saline at all concentrations. Control tissues received only the vehicle.

**Isolated Vessels**

**Preparation.** After the airway excision, the tissues were transferred to a refrigerated dissection dish filled with cold (10°C) Krebs-Ringer bicarbonate buffer. Segments of bronchial artery (~100–300 \mu m in diameter, 2–3 mm in length) were then dissected from the lobar bronchi. The vessels were mounted as rings in a dual-wire myograph (Myograph Systems 3, J.P. Trading, Aarhus, Denmark) and bathed in warm (37°C) Krebs buffer that was continually gassed with 95% O_2-5% CO_2.

Once the vessel rings were mounted in the myograph, arterioles were normalized to an internal circumference (IC) of 0.9 \times IC_{100}, where IC_{100} is the vessel circumference attained when fully relaxed and under a distending pressure of 100 mmHg (15). At this pressure, the arterioles are expected to develop near-maximal active wall tension. The normalized vessels were then allowed to stabilize for 60 min.

**Experimental protocol.** Vessel rings were preconstricted with either 10^{-8} M norepinephrine (NE) or 10^{-8} M vasopressin (VP), washed, and allowed to stabilize for 30 min. This priming cycle was repeated (typically 1–2 times) until the tension response was consistent. After the final priming sequence, the preconstrictor was added, and a dose-response relationship with furosemide was determined.

The effects of furosemide on porcine isolated vessels were evaluated in two groups. One group was preconstricted with 10^{-4} M NE, whereas the other was preconstricted with 10^{-8} M VP. Incremental concentrations of furosemide (from 10^{-8} to 3 \times 10^{-3} M) were then added to the organ bath in each group. The furosemide vehicle was saline at 10^{-8} to 3 \times 10^{-6} M and N,N-dimethylformamide at 10^{-5} to 10^{-3} M. Control tissues received only the drug vehicle.

**Data Analysis**

Tissue responses to the drugs were expressed as a percentage of the initial tension

\[
\text{Response} = \left( \frac{T_X}{T_I} \right) \times 100
\]

where \(T_X\) is the tension measured after drug treatment, and \(T_I\) is the initial tension induced by vasoconstrictor substances.

All data are expressed as means ± SE of three to six observations. Number of observations (n) indicate the number of tissues, each from a different animal, used in the study. A one-way ANOVA was used to compare treatment groups at all concentrations of furosemide, and \(P < 0.05\) was accepted as the level of statistical significance.

**Solutions and Drugs**

Krebs solution was composed of (in mM) 112.0 NaCl, 4.7 KCl, 2.5 CaCl_2, 2.5 MgSO_4, 1.2 KH_2PO_4, 11.6 glucose, and 25.0 NaHCO_3. All drugs were purchased from Sigma Chemical (St. Louis, MO). Stock solutions of all drugs were freshly prepared immediately before use. Stock solutions of furosemide were made in either saline or N,N-dimethylformamide and then further diluted with Krebs solution to the appropriate concentration. Isoproterenol, NE, and VP stock solutions were made with Krebs solution.

**RESULTS**

**Effect of Furosemide on Bronchial Smooth Muscle**

The effect of stepwise increments in furosemide concentration on tension development in porcine bronchial smooth muscle rings (n = 3) is shown in Fig. 1. Furosemide (10^{-8} to 10^{-3} M) did not cause relaxation of bronchi preconstricted with acetylcholine (10^{-5} M), although increased tension did occur in both control and furosemide-treated tissues due to the vehicle. Isoproterenol, NE, and VP stock solutions were made with Krebs solution.

![Log furosemide concentration vs. percent initial tension](image)

**Fig. 1.** Effect of furosemide on acetylcholine-preconstricted bronchial smooth muscle. Values are means ± SE. Responses to furosemide are normalized to the initial tension induced by 10^{-5} M acetylcholine. ○, Effect of furosemide (n = 3); ●, effect of the vehicle (n = 3). For concentrations of furosemide <10^{-5} M, the vehicle was saline. For 10^{-5} M and higher concentrations, N,N-dimethylformamide was the vehicle. No relaxant effect was observed with furosemide at any dose, and a small increase in tension occurred in both control and furosemide-treated tissues due to the vehicle.
Effect of Furosemide on Bronchial Artery Smooth Muscle

When vessels were preconstricted with $10^{-5}$ M acetylcholine (Fig. 3), furosemide caused significant relaxation of bronchial arteries at concentrations of $10^{-2}$ to $3 \times 10^{-1}$ M. Similarly, furosemide, at concentrations of $10^{-3}$ to $3 \times 10^{-1}$ M, also caused significant relaxation in vessels that were preconstricted with $10^{-8}$ M vasopressin (Fig. 4). A small relaxation response was also observed in control tissues (Figs. 3 and 4), apparently due to accumulation of the $N,N$-dimethylformamide vehicle.

DISCUSSION

The aim of this study was to determine whether furosemide exerts a differential effect on contractility of bronchial smooth muscle and bronchial arterial smooth muscle. We observed that furosemide relaxed bronchial arterial smooth muscle, supporting the previous supposition that the antiasthmatic properties of furosemide are related to vasodilation and increased blood flow to the airways (9). Conversely, we found that porcine bronchial smooth muscle was unresponsive to furosemide but relaxed in response to the $\beta$-adrenergic-receptor agonist isoproterenol. We conclude that furosemide, under the experimental conditions of this study, does not cause relaxation of preconstricted pig bronchi. These data therefore suggest that the antiasthmatic activity of furosemide is probably not mediated by airway dilation.

In a previous study, we showed that furosemide, when topically applied in vivo to the adventitial surface of the rat trachea, dilates tracheal arterioles and venules by a nitric oxide- and cyclooxygenase-independent mechanism (5). This technique is powerful, but it has shortcomings inherent to in vivo preparations. For instance, when judging the effects of an exogenously
applied vasoactive substance on vessel diameter, it is difficult to assess the importance of peripheral influences such as alterations in intravascular pressure and endogenously released autacoids such as neurotransmitters, hormones, and inflammatory mediators. In the present study, furosemide was applied to isolated bronchial vessels to minimize the influence of these peripheral factors. These results confirmed our previous in vivo findings (5) that furosemide is indeed a dilator of the airway vasculature.

The precise mechanism by which furosemide protects the airways against asthmogenic challenges is unknown. Because this agent is effective against a variety of indirect bronchoconstrictor stimuli such as allergens, nebulized distilled water, exercise, cold air, sodium metabisulfite, AMP, and bradykinin, the antiasthmatic properties of loop diuretics must depend on a mechanism common to all stimuli. Gilbert and co-workers (9) first demonstrated that furosemide reduced the transairway thermal exchange gradient that develops during hyperpnea and suggested that this agent increased blood flow to the airways by dilating the airway vasculature. This action of furosemide would reduce airway smooth muscle reactivity by minimizing the thermal insult associated with hyperpnea. Unfortunately, this theory does not explain how furosemide protects against allergenic and chemical insults. Conversely, furosemide-induced dilution could enhance airway blood flow and increase the clearance of exogenously administered bronchoprovocants and endogenously released inflammatory mediators. This hypothesis is supported by the study of Csete et al. (6), which showed that the antigen-induced airway obstruction in allergic sheep was influenced by trancheal blood flow. The bronchoconstrictor mediators released by mast cells after stimuli such as exercise, distilled water, and allergens, as well as inflammatory substances released through sensory nerve stimulation, could therefore be rapidly cleared by the increased microcirculatory flow, minimizing their effects on airway smooth muscle.

In addition to its actions on vascular smooth muscle, it is possible that the antiasthmatic properties of furosemide are related to inhibition of liquid and mucus secretion from submucosal glands. Inflammatory autacoids, such as neurokinins and bradykinin, stimulate secretion of glandular mucus and liquid (22), and this process could contribute to airway obstruction in asthma. Furosemide, which blocks transepithelial Cl− secretion through inhibition of Na+−K+−2Cl−cotransport, could block a large fraction of this glandular volume secretion and thereby reduce the severity of airway obstruction. Indeed, bumetanide, a related loop diuretic, has been shown to inhibit acetylcholine-induced liquid secretion in porcine bronchi by 70% (26).

It has been suggested that the capacity of inhaled furosemide to reduce the airway response to indirect bronchoconstrictor stimuli may result indirectly through release of bronchodilator prostaglandins from airway epithelium (13) and renal tubular epithelium (14). The airway epithelium is an important source of PGE2 (21), an agent that has been shown to protect against nebulized distilled water and allergen-induced bronchoconstriction in asthmatic patients (18, 19). However, as suggested by Chung and Barnes (4), it is difficult to conceive why PGE2 would have a preferential protective effect against indirect challenges such as allergens, hyperpnea, and nebulized distilled water but not against direct airway constrictors such as methacholine. Additionally, the vasodilation response to furosemide in airway (5) and pulmonary (10) circulations has been shown to be cyclooxygenase and nitric oxide independent.

If the antiasthmatic properties of furosemide are related to dilation of airway vasculature, then furosemide must reach concentrations that approach 10−4 M within the airway interstitium. The inhaled furosemide aerosol must deposit in the airway surface liquid, diffuse across the airway epithelium, and distribute into the interstitial space, which contains the vasculature. Because information on bioavailability of inhaled loop diuretics is currently lacking, it is not possible to estimate the interstitial concentrations of this drug. In an in vitro study of the bioelectric properties of canine tracheal epithelium, 10- to 100-fold higher luminal doses of furosemide were required to produce equivalent effects to adventitial administration, suggesting that the airway epithelium provides a significant barrier to the diffusion of these drugs (27). However, Gilbert and co-workers (9) report that furosemide aerosols increase the delivery of heat to the airways, providing indirect evidence that the inhalation of this drug is sufficient to increase airway blood flow. The restrictive barrier properties of the airway epithelium could contribute to the differential potency of loop diuretics on protection against asthma and explain why aerosolized bumetanide, a more potent loop diuretic, is less protective than furosemide against asthma (17).

Endothelia and epithelia are known to release factors that influence smooth muscle tone. However, we do not believe that such factors play a significant role in the response to furosemide. Our previous studies with rat tracheal arterioles demonstrated that the dilatory responses to furosemide were preserved in the presence of nitro-L-arginine methyl ester, an inhibitor of nitric oxide synthesis, and in the presence of indomethacin, a cyclooxygenase inhibitor (5). Although we cannot discount the possibility of species differences, these findings suggest that furosemide does not induce dilation through nitric oxide or prostaglandin release from endothelial cells. One could argue that the absence of a response to furosemide was related to damage to the bronchial epithelium, which might be required to release a relaxing factor. This is unlikely to be the case. In a previous study of pig bronchi, our laboratory found that the surface epithelium of this tissue was very resistant to abrasion and was completely removed only after vigorous rubbing with a rough, wooden ream (1). Therefore, the epithelium of the tissues used in the present study was certainly intact. Furosemide might have induced an observable
dilator response in bronchi if it was added before, rather than after, acetylcholine. We believe that this is unlikely, however, because furosemide did not cause any measurable effect on airway tension at even supramaximal (1 mM) concentrations.

In summary, we provide evidence from this study on pigs that furosemide is a selective dilator of vascular but not airway smooth muscle. Although the precise mechanism by which furosemide protects against an asthmatic challenge remains to be determined, these data support previous contentions that the antiasthmatic properties of this agent are related to changes in airway vascular hemodynamics (9).

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