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Effect of furosemide on the response of laryngeal receptors to low-chloride solutions

F.B. Sant'Ambrogio, G. Sant'Ambrogio, J.W. Anderson

Effect of furosemide on the response of laryngeal receptors to low-chloride solutions. F.B. Sant'Ambrogio, G. Sant'Ambrogio, J.W. Anderson. ©ERS Journals Ltd 1993.

ABSTRACT: Laryngeal irritant receptors are stimulated by water and solutions lacking chloride ions, such as isotonic dextrose. It has been reported that furosemide (frusemide) reduces cough evoked by inhalation of low-chloride solutions.

We studied the effect of furosemide on the response of laryngeal receptors to isotonic dextrose. Experiments were performed on nine dogs anaesthetized, spontaneously breathing through a tracheostomy, and with the upper airway functionally isolated. We recorded the activity of 13 laryngeal irritant receptors. Isotonic dextrose (4 ml) was instilled into the laryngeal lumen, before and after administration of a furosemide solution (3.75 mg·ml^{-1}) into the upper airway.

Before furosemide, dextrose increased the activity of the 13 receptors from 1.0±0.5 to 25.0±3.5 impulses (imp)·s^{-1} (average discharge in the first 10 s of activation) and, 1–2 min after furosemide, from 0.3±0.2 to 13.4±3.2 imp·s^{-1}; the difference between the stimulation by dextrose before and after furosemide was statistically significant. In contrast, the response to distilled water of four respiratory-modulated mechano-receptors (known to be activated by low-osmolality solutions) was not modified by furosemide.

These results suggest that the furosemide-mediated inhibition of cough induced by inhalation of low-chloride solutions is, at least in part, due to the inhibitory effect of this substance on irritant receptor stimulation.

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Dept of Physiology and Biophysics, The University of Texas Medical Branch, Galveston, USA.

Correspondence: F.B. Sant'Ambrogio
Dept of Physiology and Biophysics
The University of Texas Medical Branch
Galveston
TX 77555-0641, USA.

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The larynx is supplied with afferents that represent several sensory modalities. In recent years [1, 2], we have characterized these endings as flow/cold receptors, pressure receptors, drive receptors (stimulated by the contraction of intrinsic laryngeal muscles and/or by tracheal tug), and irritant type receptors (activated by mechanical and chemical irritants). With the notable exception of flow/cold receptors, the other types of endings were found, in a different proportion, to be stimulated by water and water solutions [2]. The water response of pressure and drive receptors, hereby defined as respiratory-modulated slowly-adapting mechanoreceptors, is due to the hypo-osmolality of the solution, whereas that of irritant receptors is distinctly due to the lack of chloride anions. In fact, isosmotic solutions of dextrose or sodium gluconate, are consistently capable of activating these endings [2]. Irritant receptors, which have endings in close proximity to the airway epithelium, respond to known tussigenic stimuli, and are thus deemed to be responsible for the cough elicited from the larynx [3, 4].

Inhalation of low-chloride solutions elicits apnoea in newborn dogs [5], and cough in humans [6, 7]. In a recent study on normal subjects, VENTRESCA *et al.* [8] found that cough elicited by aerosols of low-Cl^- solutions is attenuated by previous inhalation of furosemide (frusemide), a loop diuretic, that also alters ionic flux in other epithelial cells and in neuronal tissue by inhibiting the Na^+-2Cl^--K^+ co-transport system [9].

We worked to ascertain whether furosemide inhibits the response of laryngeal irritant receptors to low-chloride solutions, supporting, therefore, the existence of a cause-effect relationship between activation of these endings and laryngeal cough.

Methods

Experiments were performed on nine mongrel dogs of either sex, weighing 10–15 kg. They were sedated with an intramuscular injection of ketamine (10 mg·kg^{-1}) and anaesthetized with a mixture of α-chloralose (50 mg·kg^{-1}) and urethane (500 mg·kg^{-1}), injected intravenously.

The animals were placed supine on an operating table. The trachea was exposed in the neck and cut longitudinally to introduce a cannula with three sidearms (fig. 1) that allowed the diversion of breathing from the upper airway to the tracheostomy, and the possibility of functionally isolating *in situ* the upper airway [10]. A large polyethylene tube (I.D. 8 mm) was introduced orally, and positioned with its tip just below the epiglottis, facing the larynx.

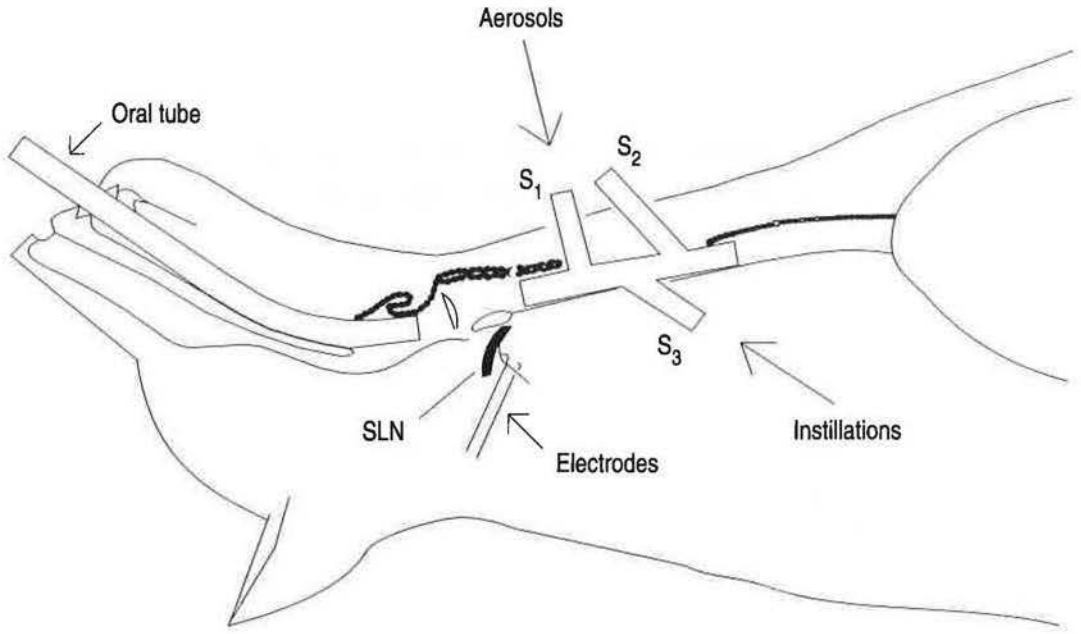

Fig. 1. – Experimental set-up. When aerosols were administered, the upper airway was functionally isolated by inflating the cuff of a Foley catheter, introduced through S_3, between S_1 and S_2. See text for further details. SLN: superior laryngeal nerve.

Polyethylene catheters were introduced into the femoral artery and vein to monitor arterial blood pressure and to administer additional doses of anaesthetics, respectively. Another polyethylene catheter filled with saline was introduced transorally into the oesophagus, to measure intrathoracic pressure.

The superior laryngeal nerves (SLN) were isolated, and their internal branch cut at the junction with the external branch. A virtually total deafferentation of the larynx is necessary to avoid reflex responses that would secondarily modify receptor activity. The peripheral cut end, usually that of the right SLN, was placed on a dissecting tray filled with paraffin oil, and desheathed, under a dissecting microscope, with the aid of iridectomy scissors and watchmaker forceps. Single unit action potentials were recorded from thin filaments dissected out of the nerve; the signals were amplified, displayed on an oscilloscope and recorded on an electrostatic recorder (Gould ES 1000) together with oesophageal pressure.

One-three receptors were studied in each dog. Irritant receptors were identified by their scant and random baseline activity, and their rapidly adapting response to a maintained mechanical stimulation (*e.g.* inflation of the cuff of a Foley catheter into the laryngeal lumen) and to solution lacking Cl^- ions. Slowly-adapting respiratory-modulated mechanoreceptors were recognized by the relationship of their discharge to the breathing cycle, the slow adaptation to a maintained mechanical stimulus and the response to distilled water. Irritant receptors are stimulated by solutions lacking Cl^-, whilst respiratory-modulated receptors are stimulated by hypo-osmolal solutions; moreover, the delay and the time to peak activation is much shorter for irritant than for respiratory-modulated, slowly-adapting receptors [2].

Experimental protocol

Receptors were challenged with iso-osmolal (280–310 mOsm) solutions of dextrose (irritant receptors) or distilled water (respiratory-modulated receptors) at 37°C; most of the receptors were also challenged with 0.9% saline at 37°C. Four millilitres of the challenging solutions were instilled into the laryngeal lumen *via* a catheter with multiple holes in the last 2 cm of its length. A furosemide solution (3.75 $mg \cdot ml^{-1}$ in 0.9% sodium chloride) was then aerosolized (DeVilbiss ultrasonic nebulizer) through the upper airway, in the expiratory direction (fig. 1), for 8 min. In a few instances, 5 ml of furosemide was instilled, in-stead. Instillation of the dextrose solution was then repeated after 1–2 min from furosemide administration and, for eight endings, also at successive intervals ranging from 6–45 min. After each instillation, the laryngeal lumen was flushed with warm (≈37°C) saline, that was then removed by suction.

Data analysis

Irritant receptors. Action potentials were counted in control (before the challenge) and test (after the challenge) conditions. The average discharge (impulses (imp)$\cdot s^{-1}$) of the receptors in the 10 s preceding the challenge was considered as control. Control activity was compared to the average discharge in the first 10 s from the introduction of the challenge; most of the stimulatory effect subsided within 10 s. Control and test data for the saline challenges, and the dextrose challenges before and after furosemide aerosolization were compared, using analysis of variance; multiple comparisons were performed

with the Scheffé test [11], using a commercially available computer program (ABstat release 6.52, by AndersonBell).

Respiratory-modulated mechanoreceptors. Action potentials were counted for three breaths in control conditions and during steady-state activation by water; the average rate of discharge (imps·s^{-1}) was then calculated. Differences in the rate of discharge between control and test, and changes in receptor activation before and after furosemide, were evaluated using a paired t-test. Changes were considered statistically significant if p was <0.05. Data are presented as mean±SEM.

This study was performed in accordance with the public health service (PHS) Policy on Humane Care and Use of Laboratory Animals, the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Act (7 U.S.C. *et seq.*). The animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC protocol No. 89-09-203) of The University of Texas Medical Branch at Galveston.

Results

Laryngeal irritant receptors

Thirteen rapidly-adapting laryngeal receptors were studied. Their rate of discharge in control conditions was 1.0±0.5 imp·s^{-1} and after instillation of iso-osmolal dextrose rose to 25.0±3.5 imp·s^{-1} (average of the first 10 s of stimulation; p<0.001). Furosemide was then aerosolized or instilled into the upper airway. This procedure did not significantly alter the baseline activity of the receptors (0.3±0.2 imp·s^{-1}), but reduced the stimulatory effects of the dextrose solutions in 11 out of 13 endings (fig. 2). The average activation by dextrose of the 13 receptors was now 13.4±3.2 imp·s^{-1}, still significantly different from control (p=0.006). The difference in the stimulatory effects of the dextrose solutions before and after furosemide was statistically significant (p=0.019) (fig. 3). Saline instillation did not significantly alter receptor activity (control 1.0±0.3 imp·s^{-1}, test 3.1±1.3 imp·s^{-1}, n=10).

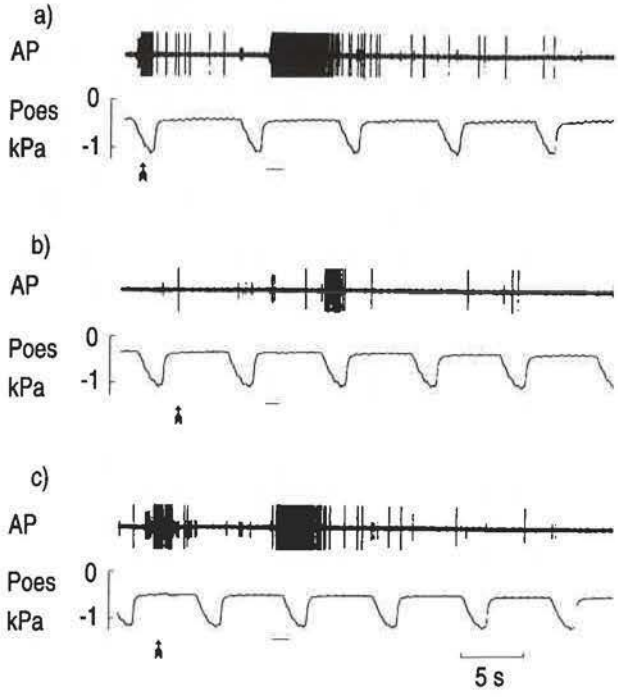

Fig. 2. – Response of a laryngeal irritant receptor to an iso-osmolal solution of dextrose: a) in control conditions; b) 1 min after furosemide; and c) during recovery (25 min after furosemide). AP: action potentials; Poes: esophageal pressure. Arrows indicate time of insertion of catheter (which often mechanically stimulates the ending). The horizontal lines indicate time of instillation of dextrose.

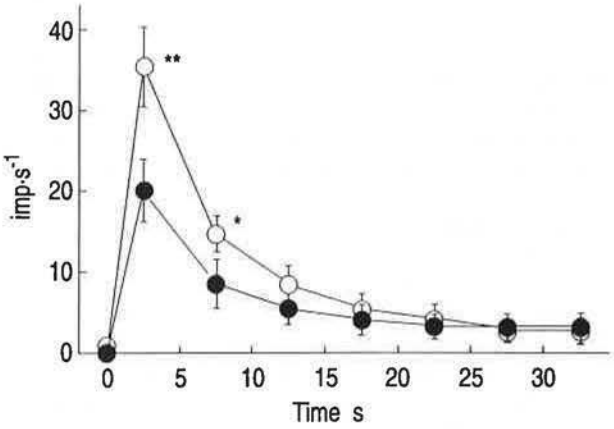

Fig. 3. – Time course of the effect of iso-osmolal solutions of dextrose on the 13 laryngeal irritant receptors tested, before (○) and after (●) aerosolized furosemide. The receptor discharge in control conditions is represented at time 0. **: p<0.001; *: p<0.05. Each point represents the average of the mean discharge of 13 receptors, calculated in bins of 5 s. imp: impulses.

For eight of the receptors, in which activation by dextrose was reduced after furosemide aerosols, challenges were repeated at intervals varying from 6–45 min (mean 24.3±4.2 min), in order to evaluate their recovery time. Once a receptor had reached approximately 80% of the pre-furosemide activation, no further challenges were performed. For these endings, the increase in activity due to dextrose before furosemide was 27.0±4.4 imp·s^{-1}, 1 min after furosemide 11.1±4.3, and during recovery 20.8±2.6 imp·s^{-1} (at a mean time of 24.3±4.2 min) Only the values before and immediately after furosemide were significantly different (p=0.042).

Laryngeal respiratory-modulated receptors

Four slowly-adapting respiratory-modulated receptors were tested. Water instillation before furosemide increased receptor activity from 9.0±0.9 to 32.4±4.8 imp·s^{-1} (p=0.020) and after furosemide from 7.3±2.6 to 31.5±7.5 imp·s^{-1} (p=0.049). The increases in activity that followed water instillation before and after furosemide were not significantly different (p=0.478).

Discussion

Water is a potent laryngeal irritant; when instilled into the larynx of newborn and adult anaesthetized animals, it

induces bradycardia and markedly reduces ventilation, even to the point of a protracted apnoea [5, 12, 13]. In anaesthetized sleeping dogs, SULLIVAN *et al.* [14] observed either arousal followed by cough or a brief apnoeic response, swallowing and bradycardia, depending on the sleep stage. BOGGS and BARTLETT [5] ascribed the effect of water to the lack of chloride ions and not to hypo-osmolality; in fact, any iso-osmolal solution tested could elicit apnoea, as long as the concentration of Cl^- or other small permeant anions was low.

In humans with mild asthma, inhalation of distilled water mists causes cough and bronchoconstriction; bronchoconstriction is caused by alteration in osmolality away from iso-osmolality, and cough is elicited by solutions lacking permeant anions [6]. Inhaled lidocaine inhibits cough, but not bronchoconstriction, indicating that cough is not merely a secondary response to smooth muscle contraction [15]. These findings might also suggest that bronchoconstriction elicited by water is mediated by respiratory modulated mechanoreceptors, more deeply located in the airway wall and, therefore, less likely to be affected by lidocaine, and stimulated by hypo-osmolal solutions [2]. On the other hand, cough should be mediated by the more superficially located irritant receptors, which are activated by low chloride solutions [2]. Moreover, these results would also indicate that the cough reflex originates mainly from the larynx and/or large, central tracheobronchial airways, where irritant type endings have indeed been found in greater concentration [16, 17]. VENTRESCA *et al.* [8] showed that furosemide attenuates the cough induced by low chloride content solutions, but not that elicited by inhaled capsaicin. These findings suggest the presence of two separate afferent pathways for the cough reflex.

Our study shows that furosemide, used at the same concentration and by the same method of delivery as used in the work by VENTRESCA *et al.* [8], strongly diminishes the stimulatory effect of low chloride solutions on laryngeal irritant receptors. BOGGS and BARTLETT [5] hypothesized that a decreased concentration of Cl^- in the external environment of the endings could result in a loss of internal Cl^- that, in the absence of other permeant anions capable of replacing them, could lead to depolarization of the nerve endings. In the presence of furosemide, an inhibitor of Cl^- transport, the loss of internal Cl^- would occur to a much lesser extent resulting, therefore, in a weaker stimulation.

Furosemide failed to alter the response to dextrose in 2 of the 13 irritant receptors tested; in both cases, even additional furosemide administrations (either aerosolized or instilled) remained without effect. Whereas one of the two endings was the only one found in an individual dog, the other irritant not affected by furosemide was studied in another animal, in which two other receptors were affected by furosemide; we have not been able to find a convincing explanation for the different behaviour.

The variability of the time required to achieve a good recovery from the inhibitory effect of furosemide on the response to dextrose, might reflect differences in blood supply to the microenvironment of the afferent endings. In any event, our results are consistent with findings indicating that the inhibitory effect of furosemide lasts at least 20 min [8].

We did not find any effect of furosemide on the baseline activity of laryngeal receptors; this observation is in agreement with a study in cats in which no effect of furosemide was found on the spontaneous activity of tracheobronchial irritant receptors [18]. The lack of any direct effect of furosemide on the baseline activity of the endings is also in line with results from studies on neuronal tissue. In fact, no effect of furosemide was reported on membrane potential or characteristics of action potentials during resting activity in ganglion cells of *Aplysia* [19, 20], and cat dorsal root ganglia [21]. NAKAI *et al.* [20] postulated that this substance, in neuronal tissue, has a blocking effect only on the open state of Cl^- channels. This could explain the presence of a furosemide effect only when the receptor is stimulated.

It is interesting to note that irritant receptors in the intrapulmonary bronchi are stimulated by non-isotonic solutions, rather than solutions of low Cl^- concentration [22], whereas in the trachea both types of irritant endings have been demonstrated [23]. This suggests that the effect of furosemide on low-chloride solution-induced cough is mediated through the inhibition of laryngeal irritant receptors, and possibly also by those irritant receptors located in the extrathoracic trachea, which are similarly responsive to low-Cl^- solutions.

ESCHENBACHER *et al.* [6] suggested that bronchoconstriction due to water inhalation depends on the hypo-osmolal properties of water. Irritant receptors in the intrathoracic airway [22], and some in the extrathoracic trachea [23], are not stimulated by low-chloride containing solutions, but are instead responsive to hypo-osmolal solutions, suggesting that receptors stimulated by low osmolality have a role in eliciting a bronchoconstrictive response. ROBUSCHI *et al.* [24] reported that furosemide was effective in preventing bronchoconstriction induced by water inhalation. This type of bronchoconstriction is supposedly due to a decrease in osmolality of the airway surface liquid [6].

We have found no effect of furosemide on the response to water of laryngeal respiratory-modulated mechanoreceptors stimulated by low osmolality, in line with the notion that furosemide acts through its effect on the Na^+-$2Cl^-$-K^+ co-transport mechanisms at the neuronal membrane level and not through mechanisms related to osmolality [20, 25]. Possibly, in the experiments of ROBUSCHI *et al.* [24] the inhibitory effect of furosemide was not mediated through attenuation of receptor activity. Alternatively, receptor characteristics vary among species or, in asthmatics, the properties of the receptors may be altered.

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