Inhaled Furosemide Inhibits Behavioral Response to Airway Occlusion in Anesthetized Cats

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Background: A recent study showed that inhaled furosemide greatly improves experimentally induced dyspnea in humans. The objective of the current study is to test the hypothesis that inhaled furosemide suppresses the behavioral response to airway occlusion without changing the behavioral response to a somatic noxious stimulus in anesthetized animals.

Methods: In 10 spontaneously breathing cats anesthetized with isoflurane, anesthetic ED_{50} was determined by measuring an end-tidal anesthetic concentration while observing escape behavior. The monitored behavior consisted of purposeful movement of the head and forearm after endotracheal tube occlusion. The duration from the start of airway occlusion to the onset of the positive response (DOCCL) was measured at the highest concentration of isoflurane permitting the positive motor response to airway occlusion before pretreatment. ED_{50} values (minimum alveolar concentration) for the suppression of a somatic motor response to a noxious stimulus induced by toe pinch (toe-pinch ED_{50}) were also determined. Then, the effects of inhaled furosemide or vehicle on the ED_{50} for the suppression of the behavioral response to airway occlusion, DOCCL, and toe-pinch ED_{50} were evaluated in a randomized, cross-over design.

Results: The ED_{50} for the suppression of the behavioral response to airway occlusion significantly decreased ($P < 0.01$) and DOCCL was significantly prolonged ($P < 0.01$) after furosemide inhalation, whereas vehicle inhalation did not change these measurements. The decrease in ED_{50} for the suppression of the behavioral response to airway occlusion after furosemide inhalation lasted 3 h. Furosemide inhalation did not affect the toe-pinch ED_{50}.

Conclusion: Inhaled furosemide suppressed the behavioral response to airway occlusion in anesthetized animals without affecting the response to somatic noxious stimulus. The authors' animal model of respiratory distress may be applicable to the study of dyspnea in regard to its mechanism and treatment.

DYSPNEA, like pain, evokes strong emotional and behavioral responses. Despite recent advances in the study of dyspnea,[1] its genesis and pathophysiology remain unclear. One problem associated with the study of dyspnea is that there is no adequate experimental animal model. In previous studies, we analyzed the escape behavior (characterized by vigorous movement of the head and extremities in response to complete airway obstruction) in lightly anesthetized cats and introduced a new concept of an ED_{50} for an inhalational anesthetic for the suppression of behavioral response to airway occlusion.

This enabled us to assess the contribution of pulmonary vagal afferents to the genesis of distress and dyspnea.[2,3] Using this model of respiratory distress, it may also be possible to assess various treatments for dyspnea. We have recently reported that inhaled furosemide can improve experimentally induced dyspnea.[4] We have also suggested that this improvement might be due to alteration in sensory receptor function in the airway epithelium and its vicinity.[5]

If our animal model is useful for assessing various treatments of dyspnea, we would expect that inhaled furosemide would suppress the behavioral response to airway occlusion without changing the behavioral response to somatic noxious stimuli. We reasoned that if dyspnea and escape behavior from respiratory stress are related, inhalation of furosemide would affect both.

To test this hypothesis, we examined the effects of inhaled furosemide on the ED_{50} concentration of isoflurane for the suppression of the behavioral response to airway occlusion (airway occlusion ED_{50}), the duration from the start of airway occlusion to the onset of positive behavioral response (DOCCL), and the ED_{50} values (minimum alveolar concentration) for noxious stimulus induced by toe pinch (toe-pinch ED_{50}).

Methods

All surgical procedures and experimental protocols were approved by the Animal Care and Use Committee of Chiba University School of Medicine (Chiba, Japan). Ten adult cats (seven males and three females) weighing 3.9 ± 1.1 kg (mean $\pm$ SD) were anesthetized with isoflurane. After tracheal intubation, each cat was placed in the right lateral position, and an endotracheal tube was connected to a non-rebreathing circuit *via* a three-way stopcock with two side ports. The cat was allowed to breathe spontaneously while anesthesia was maintained with 1–3% isoflurane in oxygen. Airway pressure was measured continuously with a transducer at a side port of the three-way stopcock. Airway gas was sampled at a total flow rate of 27 ml/min from another side port for continuous measurements of carbon dioxide, oxygen, and isoflurane concentrations using a gas analyzer (1H21A; Acoma, Tokyo, Japan) and an anesthetic gas monitor (model 303; Atom, Tokyo, Japan) connected in series. The right femoral vein was cannulated for continuous infusion of acetated Ringer's solution with 5% dextrose. The right femoral artery was also cannulated for measurements of arterial blood pressure and for withdrawal of blood gas samples for analysis using a blood

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gas analyzer (288 Blood Gas System; Ciba-Corning, Tokyo, Japan). The electromyographic activities of the alae nasi were recorded with a pair of needle electrodes that were placed in parallel, 5–6 mm apart, to monitor spontaneous respiratory activity. Rectal temperature was continuously monitored and maintained at 37–38°C using a water blanket and an infrared heating lamp. Arterial blood pressure, airway pressure, end-tidal carbon dioxide, and electromyographic data were recorded simultaneously on an eight-channel chart recorder (RJG-4128; Nohon Koden, Tokyo, Japan). The study consisted of two protocols, and each protocol was conducted on 2 separate days with an interval of 12 days.

Protocol 1

This protocol was performed in a randomized, crossover design. On each of the 2 days, a set of two trials was conducted in each animal. The initial trial was always performed without pretreatment (before inhalation), and the second trial was performed after inhalation of furosemide or vehicle (after inhalation of the test solution). Using an ultrasonic nebulizer (NE-U12; Omron, Tokyo, Japan), 6 ml furosemide (Lasix; Hoechst Tokyo, Tokyo, Japan) as a 10-mg/kg solution or 6 ml vehicle (the diluent solution without furosemide contains NaCl 7.0 mg, NaOH to reach pH 9, and water to make up 1 ml) was delivered for 10 min from the inhalation side of the breathing circuit.

In each animal, the ED_{50} required to suppress the behavioral response to airway occlusion before inhalation of the test solution was determined by measuring end-tidal anesthetic concentrations of isoflurane while observing escape behavior consisting of the purposeful movement of the head and forearm as described in our previous studies.[2,3] In brief, after maintaining a predetermined end-tidal concentration of isoflurane for 30 min, airway occlusion was performed at end expiration by turning the three-way stopcock in the breathing circuit and was maintained maximally for 6 min. The end-tidal concentration of isoflurane was increased by 0.1–0.2 vol% if the response was positive; otherwise, the concentration was decreased in the same fashion, and the airway occlusion was repeated. During this procedure, we measured the duration from the start of airway occlusion to the onset of positive behavioral response (DOCCL) at the highest concentration of isoflurane permitting the positive behavioral response. Blood gas samples were also obtained before and at the time of termination of airway occlusion.

After the aforementioned measurements, the animal inhaled either furosemide or vehicle, and the value of DOCCL was measured while maintaining the same concentration of isoflurane used for the measurement of DOCCL before inhalation of the test solution. When there was no positive behavioral response to airway occlusion, the value of DOCCL was calculated as 6 min.

After this procedure, the procedures for the determination of airway occlusion ED_{50} were repeated, and the values of airway occlusion ED_{50} after inhalation of the test solution were obtained.

Protocol 2

In each animal, either the airway occlusion ED_{50} or the toe-pinch ED_{50} values were determined before inhalation of furosemide in a randomized, cross-over design with an interval of 2 days. Toe-pinch ED_{50} was determined in accordance with the bracketing procedure as described in Ide *et al.*[2] The determination of airway occlusion ED_{50} and toe-pinch ED_{50} was repeated 1 h after furosemide inhalation every 30 min for 6 h. The objective of this protocol was twofold. First, we wanted to know whether the effects of furosemide on airway occlusion ED_{50} and toe-pinch ED_{50} were similar. Second, we also wanted to know the time course of the effect of furosemide.

Data Analysis

All data are presented as mean ± SD. Statistical analysis was performed using two-way repeated analysis of variance followed by the Scheffe F test and paired *t* test, where appropriate. Differences in mean values of variables were judged to be significant if *P* was less than 0.05.

Results

Figure 1 shows changes in airway occlusion ED_{50} before and after test agents. The values of airway occlusion ED_{50} for isoflurane before furosemide inhalation (1.8 ± 0.3%) significantly decreased after furosemide inhalation (1.4 ± 0.3%; $P < 0.01$), whereas there was no significant difference between the values before and after vehicle inhalation (1.7 ± 0.3 *vs.* 1.7 ± 0.3%).

After furosemide inhalation, no animal showed any

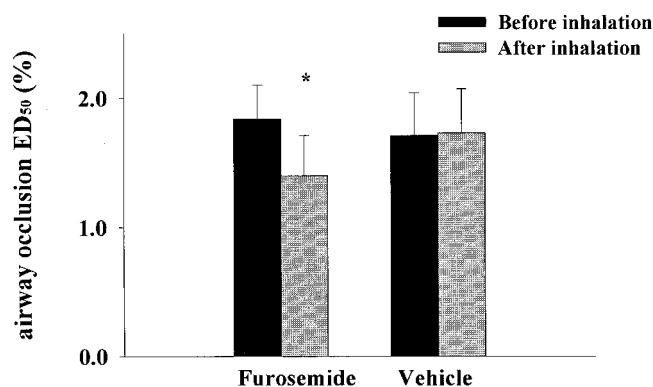**Fig. 1. Changes in ED_{50} concentration of isoflurane for the suppression of behavioral response to airway occlusion (airway occlusion ED_{50}) before and after inhalation of furosemide and vehicle. Values are mean ± SD. *$P < 0.01$ compared with the value before inhalation.**
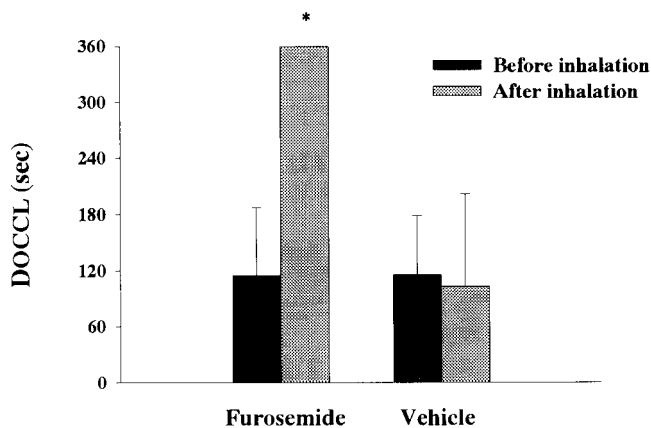
Fig. 2. Average values of the duration from the start of airway occlusion to the onset of the positive motor response (DOCCL) before and after inhalation of furosemide and vehicle. Values are mean ± SD. *P < 0.01 compared with the value before inhalation.
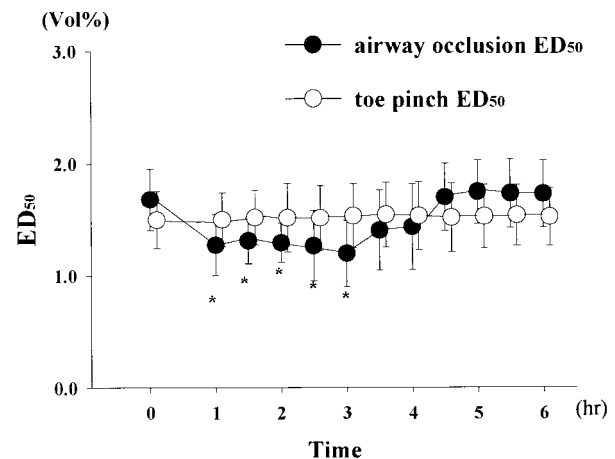

Fig. 3. Time course of changes in airway occlusion ED_{50} and toe-pinch ED_{50}. Control values were obtained before inhalation of furosemide and are represented at time 0. Values are mean ± SD; n = 10. *P < 0.05 compared with control value.

positive behavioral response within 6 min of airway occlusion, and DOCCL became 360 s in all animals. This is significantly higher than the DOCCL values of 114 ± 73 s obtained before furosemide inhalation (fig. 2). Average values of mean arterial blood pressure and heart rate, pH, arterial carbon dioxide tension ($Paco_2$), and arterial oxygen tension (Pao_2) before the start and at the termination of airway occlusion are summarized in table 1. The values of pH, $Paco_2$, and Pao_2 at the termination of airway occlusion were significantly different from those before the start of airway occlusion.

Figure 3 shows the time course of changes in airway occlusion ED_{50} and toe-pinch ED_{50} after furosemide inhalation. Furosemide inhalation immediately caused a decrease in airway occlusion ED_{50}, and the decrease in airway occlusion ED_{50} remained nearly steady for 3 h. In contrast, furosemide inhalation did not affect toe-pinch ED_{50}.

Discussion

In this study, we demonstrated that airway occlusion ED_{50} decreases and DOCCL is prolonged after inhalation

of furosemide in an anesthetized animal model. The respiratory stress during airway occlusion is probably primarily due to the increasing medullary respiratory drive, secondary to the increasing ventilatory drive. The concept of airway occlusion ED_{50} is based on the observation that when respiratory stress is noxious enough, it can evoke an all-or-none type of escape response, even in an anesthetized condition.[2,3] The measurement of DOCCL was used as a behavioral measure of the tolerable limit of respiratory stress, assuming that the onset of the positive behavioral response during airway occlusion in anesthetized animals might be comparable to the breaking point of breath-holding in conscious subjects. Our findings indicate that inhalation of furosemide suppresses the behavioral response to airway occlusion in an anesthetized condition, which is in accordance with the previous report that inhaled furosemide prolongs breath-holding time and alleviates experimentally induced dyspnea in conscious human subjects.[4] Therefore, our animal model of respiratory distress may be useful for assessing the potential treatment of dyspnea. However, dyspnea is subjective sensation, and it is impossible for us to know whether the sensation of dys-

Table 1. Average Values of pH, $Paco_2$, Pao_2, Arterial Pressure, and Heart Rate Before and at the Discontinuation of Airway Occlusion

	Before Airway Occlusion					After Airway Occlusion				
	pH	$Paco_2$ (mmHg)	Pao_2 (mmHg)	MAP (mmHg)	HR (beats/min)	pH	$Paco_2$ (mmHg)	Pao_2 (mmHg)	MAP (mmHg)	HR (beats/min)
Furosemide inhalation										
Before	7.47 ± 0.05	33 ± 6	416 ± 105	99 ± 18	174 ± 26	7.43 ± 0.09	37 ± 10	362 ± 117	99 ± 37	179 ± 22
After	7.48 ± 0.05	32 ± 7	338 ± 94	120 ± 29	162 ± 29	7.21 ± 0.11*	81 ± 24*	121 ± 65*	121 ± 27	169 ± 29
Vehicle inhalation										
Before	7.49 ± 0.03	30 ± 7	447 ± 96	114 ± 2	182 ± 27	7.4 ± 0.06	37 ± 11	409 ± 76	117 ± 25	183 ± 27
After	7.50 ± 0.05	32 ± 4	402 ± 59	107 ± 21	186 ± 29	7.4 ± 0.10	38 ± 10	343 ± 128	120 ± 21	181 ± 28

Values are mean ± SD.

* $P < 0.01$.

MAP = mean arterial blood pressure; HR = heart rate; $Paco_2$ = partial pressure of arterial carbon dioxide; Pao_2 = partial pressure of arterial oxygen.

pnea that develops during breath-holding in a conscious human subject is comparable to some sensation generated at subcortical levels in the brain of the anesthetized animal during airway occlusion. In addition, dyspnea may consist of multiple sensations mediated by different underlying mechanisms, and it is possible that different conditions may cause different types of dyspnea. Therefore, caution must be exercised in extrapolating the results obtained from our anesthetized animal model to those obtained from conscious human subjects.

Because it is unlikely that inhaled furosemide can exert direct depressant effects on the central nervous system, the observed effects of inhaled furosemide are presumably caused by peripheral mechanisms. Although afferent information from the chest wall might contribute to the generation of respiratory distress,[6-8] it is unlikely that inhaled furosemide directly affects the afferent information from the chest wall. The most plausible explanation for our findings is that inhaled furosemide can modulate pulmonary vagal afferents and thereby causes relief of respiratory distress. In our previous study, we showed that pulmonary stretch receptors are sensitized and pulmonary irritant receptors are desensitized by inhalation of furosemide, whereas intravenous furosemide causes no effect in anesthetized rats.[5] Although these observations suggest that inhaled furosemide may act directly on airway sensory receptors, the cellular mechanisms responsible for activation of pulmonary stretch receptors and pulmonary irritant receptors are unknown. However, furosemide is known to be a specific inhibitor of the $Na^+-K^+-2 Cl^-$ cotransporter in the basolateral membrane of tracheobronchial mucosa,[9] and, therefore, the changes in ion concentrations in the submucosal extracellular space within the vicinity of sensory nerve receptors are considered to be responsible for the changes in the activity of sensory nerve receptors. The results of this study are also in accordance with the results of our previous study that lung expansion prolonged DOCCL in a dose-related manner, and bilateral vagotomy abolished this effect in the same animal model,[3] suggesting the important role of pulmonary vagal afferent in relief of respiratory distress.

In this study, we also confirmed that the airway occlusion ED_{50} for isoflurane is similar to the toe-pinch ED_{50} before inhalation of furosemide.[2] This finding suggests that the degree of airway occlusion as a noxious stimulus is equivalent to that of a pinch stimulus and that the sensitivities of the neural networks involved in evoking positive behavioral responses to airway occlusion and pinch are similar at a relatively light depth of anesthesia. However, airway occlusion ED_{50} decreased, whereas toe-pinch ED_{50} did not change, after inhalation of furosemide. The dissociation of airway occlusion ED_{50} and toe-pinch ED_{50} suggests that the sensitivity of the neural networks involved in evoking the response to airway occlusion is modulated by furosemide inhalation. The finding that the decrease in airway occlusion ED_{50} lasted 3 h indicates that inhaled furosemide stays at the peripheral site and exerts its effects for a considerably long duration. This relatively long-lasting effect of inhaled furosemide has been observed in several clinical studies.[10-12]

In conclusion, our study showed that inhaled furosemide suppresses the behavioral response to airway occlusion in isoflurane-anesthetized animals, whereas furosemide inhalation did not affect the behavioral response to a noxious stimulus. Our animal model of respiratory distress may be applicable to the study of dyspnea in regard to its mechanism and treatment.

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