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Endothelin-1 in Citric Acid Aerosol Inhalation-Induced Airway Constriction of Guinea Pigs

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Abstract

Endotheline-1 (ET-1) has been shown to enhance tachykinin-induced airway constriction. This study was designed to test whether ET-1 is involved in citric acid-induced bronchoconstriction. Forty-eight anesthetized-paralyzed guinea pigs were divided into six groups of 8 animals each: saline control; citric acid; ET-1; ET-1 + citric acid; BQ123 + ET-1 + citric acid; and BQ788 + ET-1 + citric acid. BQ123 and BQ788 are specific ET_A and ET_B receptor antagonists, respectively. Each animal in the saline control group received 50 breaths of 4 ml saline aerosol and in all citric acid-treated groups was given 50 breaths of 4 ml aerosol generated from 0.6 M citric acid. In all ET-1-treated groups, each animal was exposed to aerosol generated from 10^{-8} M ET-1. The animal in the ET-1 + citric acid group was exposed to ET-1 5 min prior to the citric acid. For the last two groups, each animal was first exposed to aerosol generated from either 10^{-5} M BQ123 or 10^{-5} M BQ788. Five min later, the animal was exposed to ET-1; and then 5 min later was followed by citric acid. Dynamic respiratory compliance (Crs), forced expiratory volume in 0.1 sec ($FEV_{0.1}$), and maximal expiratory flow at 30% total lung capacity ($\dot{V}max_{30}$) were obtained before and 3-15 min after citric acid. Either citric acid or ET-1 inhalation caused significant decreases in Crs, $FEV_{0.1}$, and $\dot{V}max_{30}$, indicating airway constriction. Citric acid-induced airway constriction, for most cases, was not significantly augmented by ET-1. However, either BQ123 or BQ 788 significantly attenuated the airway constriction induced by the combination of ET-1 and citric acid. Also, in an additional study, either BQ123 or BQ788 significantly attenuated citric acid-induced airway constriction. These data suggest that endogenous ET-1 plays an important role in citric acid aerosol-induced airway constriction in guinea pigs.

Key Words: airway reactivity, acid-induced airway constriction, endothelins

Introduction

Endothelin-1 (ET-1) is a 21-amino-acid peptide and was originally isolated from vascular endothelial cells and airway epithelial cells (4, 26). Two additional isopeptides of the ET family, ET-2 and ET-3, have been identified and found to express in various tissues (14). Recent observations suggest that these peptides are released from cultured human airway epithelial cells in response to endogenous and exogenous signals (3). In addition, receptors for ETs are present on the smooth muscle of animal and human airways, and their stimulation results in airway constriction (20). ETs are among the most potent constrictors for bronchial smooth muscle in guinea pigs and humans (25). These findings suggest the possibility that ETs play an important role in the pathophysiology of asthma (15). The actions of ETs are mediated by two distinct receptor subtypes designated ET_A and ET_B receptors. Both ET_A and ET_B receptors have physiological role in ET-1-induced airway constriction in guinea pigs (22).

It is known that acid aspiration induces airway constriction (10). Also, inhalation of citric acid aerosol causes coughing (8) and plasma extravasation in the lungs (24). These effects of citric acid have been attenuated or prevented by capsaicin pretreatment to

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deplete tachykinis (9), implying that the action of citric acid may be mediated via its action on vanilloid receptors on afferent C-fibers and the subsequent release of tachykinins. Kanazawa et al. (11) reported that subthreshold concentration of ET-1 enhances capsaicin-induced airway constriction. Since both capsaicin and citric acid activates afferent C-fibers, ET-1 may also enhance the airway constriction induced by citric acid. Accordingly, the main goal of this paper was to investigate whether ET-1 enhances citric acid-induced airway constriction.

Materials and Methods

Animal Preparations

Forty-eight young Hartley strain guinea pigs weighing 200-300 g were divided into six groups of 8 animals each: saline control; citric acid; ET-1; ET-1 + citric acid; BQ123 + ET-1 + citric acid; and BQ788 + ET-1 + citric acid. BQ123 and BQ788 are specific ET_A and ET_B receptor antagonists, respectively. Following anesthesia with sodium pentobarbital (30-40 mg/kg, i. p.), each animal's trachea and jugular vein were cannulated. After being paralyzed with gallamine triethiodide (4 mg/kg, i.v.), the animal was artificially ventilated. Each animal in the saline control group received 50 breaths of 4 ml saline aerosol and in all citric acid-treated groups was given 50 breaths of 4 ml aerosol generated from 0.6 M citric acid. In all ET-1-treated groups, each animal was exposed to ET-1 aerosol (50 breaths of 4 ml aerosol generated from 10^{-8} M ET-1). The animal in the ET-1 + citric acid group was first exposed to ET-1 5 min prior to the citric acid aerosol inhalation. For the last two groups, each guinea pig was first exposed to either BQ123 aerosol (50 breaths of 4 ml aerosol generated from 10^{-5} M BQ123) or BQ788 aerosol (50 breaths of 4 ml aerosol generated from 10^{-5} M BQ788). Five min later, the animal was exposed to ET-1. Subsequently, the animal was then exposed to citric acid, 5 min following the administration of ET-1. All aerosols were generated from a nebulizer (Ultra-Neb99, DeVilbiss Co., Somerest, PA, U.S.A.).

An additional twenty-one guinea pigs were used to test whether BQ123 or BQ788 directly inhibits citric acid-induced airway constriction. These animals were divided into 4 groups: saline control (n = 3); citric acid (n = 6); BQ123 + citric acid (n = 6); and BQ788 + citric acid (n = 6). Treatment(s) of saline, citric acid, BQ123 and/or BQ788, either singly or in combination, was/were the same as those described above.

Evaluation of Bronchial Function

Each anaesthetized-paralyzed and ventilated animal was placed supine inside a whole-body plethysmograph. The flow rate was monitored with a Validyne DP45 differential pressure transducer as the pressure dropped across three layers of 325-mesh wire screen in the wall of the plethysmograph. Lung volume change was obtained via integration of flow. The airway opening pressure (Pao) or the arterial blood pressure was measured by a pressure transducer (DTX/Plus, Viggo-Spectramed, Oxnark, CA, USA) connected to a side arm of the tracheal tube or to the arterial catheter, respectively. All of the above signals were recorded on a recorder (TA11, Gould, Valley View, OH, USA). During artificial ventilation, tidal volume (V_T) and its accompanying Pao were used to calculate dynamic respiratory compliance (Crs = $V_T/\Delta Pao$). The Pao difference was measured between end-inspiration and end-expiration (ie, instance of no flow). The full maximal expiratory flow-volume (MEFV) maneuver was performed with an inflation of the lung to total lung capacity (TLC, the lung volume at Pao = 30 cmH_2O) and a subsequent deflation to residual volume with a negative pressure of 40 cmH_2O, which produces the maximal expiratory flow ($\dot{V}max$). $\dot{V}max$ at 30% baseline TLC ($\dot{V}max_{30}$) and forced expiratory volume in 0.1 second ($FEV_{0.1}$) were measured according to our previous method (16). Crs, $FEV_{0.1}$ and $\dot{V}max_{30}$ were used as indicators of airway constriction. The general experimental protocol was that the values of Crs, $FEV_{0.1}$ and $\dot{V}max_{30}$ were obtained before (baseline) and 3, 10, 15 min after citric acid aerosol inhalation.

Statistical Analysis

All values are reported as mean ± S.E. Analysis of variance was used to establish the statistical significance of differences among groups. If significant differences among groups were obtained using the analysis of variance, Bonferroni multiple range test was used to differentiate differences between groups. Differences were considered significant if $p < 0.05$.

Results

Table 1. Body Weight and Baseline Respiratory Parameters in Guinea Pigs

Group	n	BW (g)	Crs (ml/cm$H_2$0)	$FEV_{0.1}$ (ml)	$\dot{V}_{max30}$ (ml/s)
Saline control	8	262 ± 27	0.26 ± 0.04	6.9 ± 0.8	46.8 ± 9.9
Citric acid	8	242 ± 28	0.29 ± 0.09	7.4 ± 0.9	56.2 ± 12.4
Endothelin-1	8	256 ± 17	0.28 ± 0.02	7.2 ± 0.4	58.1 ± 7.9
Endothelin-1 +citric acid	8	248 ± 27	0.22 ± 0.04	6.9 ± 1.2	52.5 ± 12.5
BQ123 + endothelin-1 + citric acid	8	235 ± 21	0.22 ± 0.03	7.2 ± 0.7	62.5 ± 10.6
BQ788 + endothelin-1 + citric acid	8	251 ± 24	0.22 ± 0.04	6.9 ± 1.1	56.8 ± 12.5

Values are mean ± SE. n, the number of animals; BW, body weight; Crs, dynamic respiratory compliance; $FEV_{0.1}$, forced expiratory volume in 0.1 s; $\dot{V}_{max30}$, maximal expiratory flow at 30% baseline total lung capacity. There are no significant differences in body weight or lung functional parameters between any two groups.

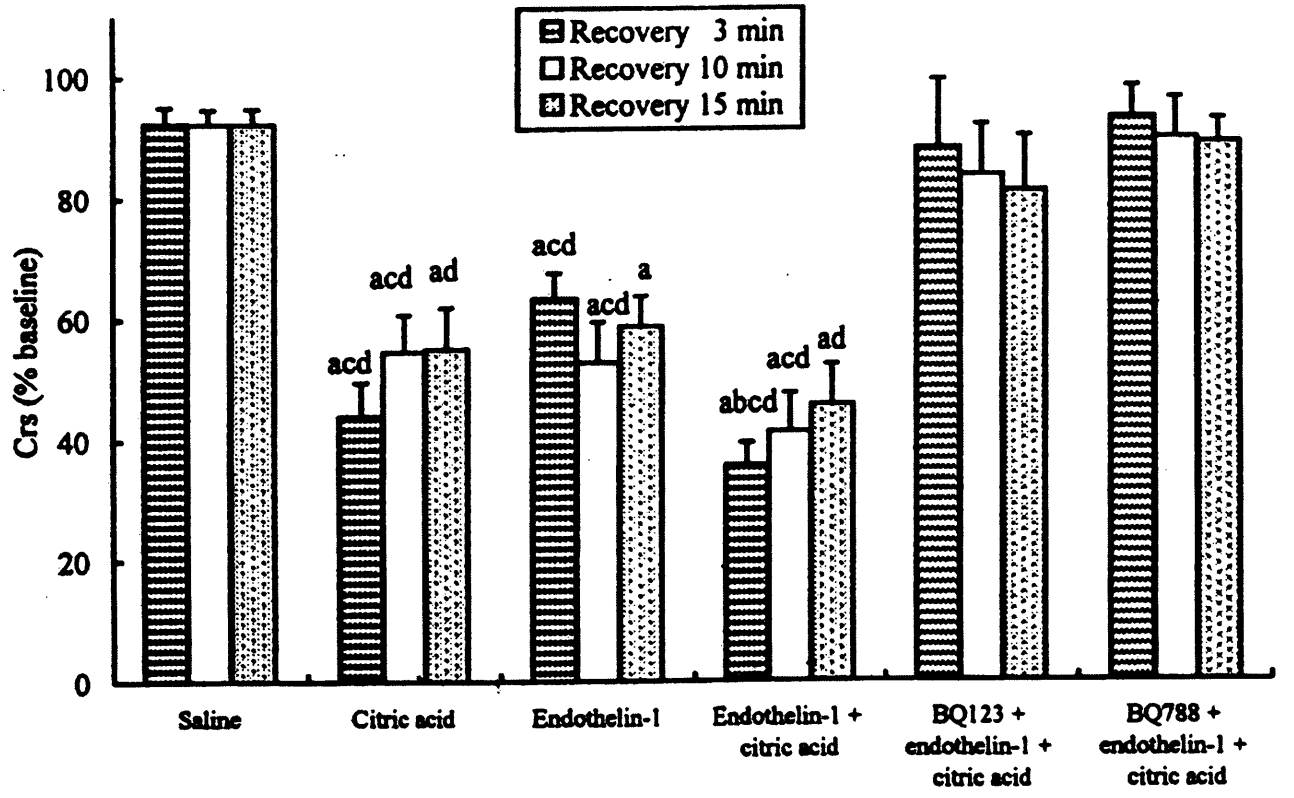

Fig. 1. Citric acid-induced alterations in dynamic respiratory compliance (Crs), expressed as percent baseline values, in six groups of 8 guinea pigs each. Statistical differences ($p < 0.05$) between groups: [a]compared to the saline control group; [b]compared to the endothelin-1 group; [c]compared to the BQ123 + endothelin-1 + citric acid group; [d]compared to the BQ788 + endothelin-1 + citric acid group.

The body weight and baseline respiratory parameters of guinea pigs are listed in Table 1. There was no significant difference in these parameters between any two groups during the baseline period. To account for individual differences, we compared the results using percent baseline values for each animal.

Effect of Citric Acid on Bronchial Function

Saline aerosol inhalation did not induce any significant alteration in Crs (Fig.1). On the other hand, citric acid aerosol inhalation caused a significant decrease in Crs, indicating severe citric acid-induced airway constriction. Similar to Crs, citric acid aerosol inhalation caused significant decreases in $FEV_{0.1}$ (Fig. 2) and $\dot{V}max_{30}$ (Fig. 3).

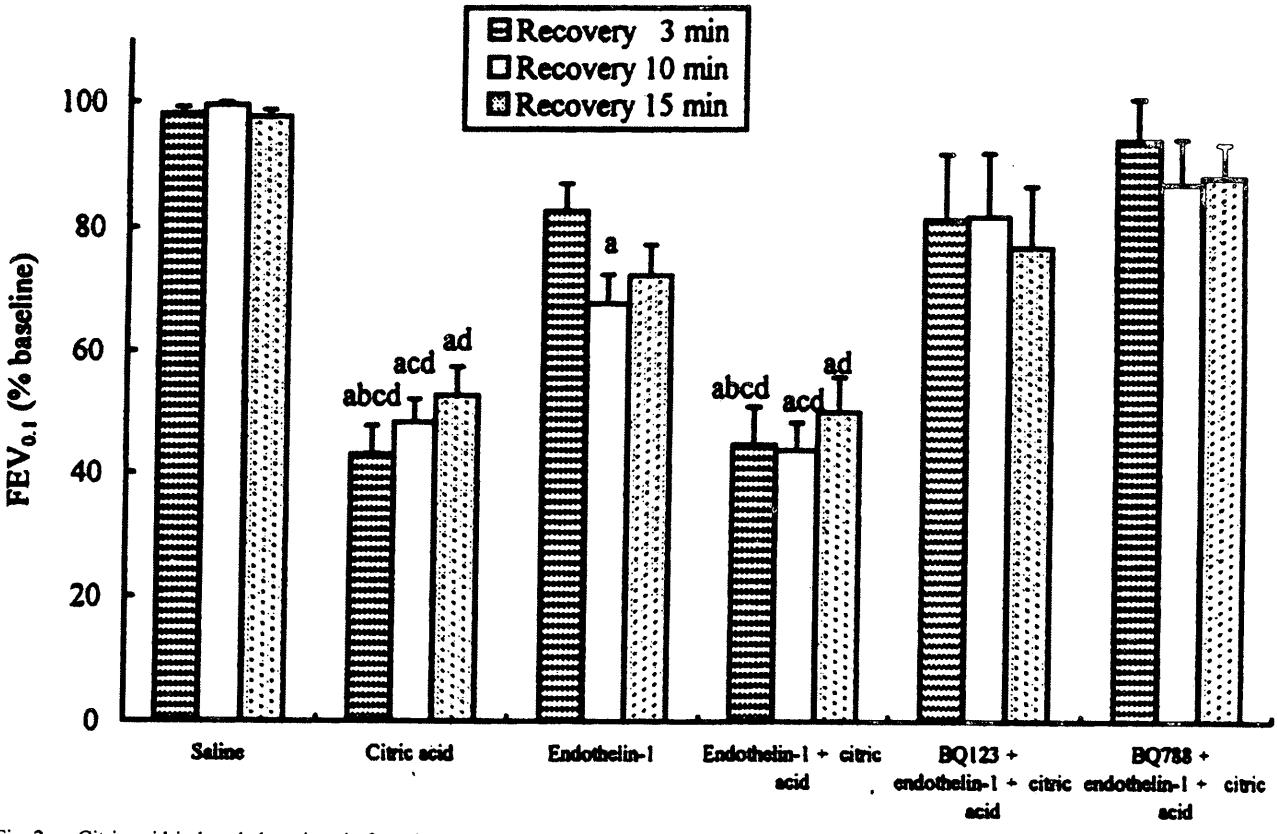

Fig. 2. Citric acid-induced alterations in forced expiratory volume in 0.1 sec ($FEV_{0.1}$), expressed as percent baseline values, in six groups of 8 guinea pigs each. Statistical differences ($p < 0.05$) between groups: [a]compared to the saline control group; [b]compared to the endothelin-1 group; [c]compared to the BQ123 + endothelin-1 + citric acid group; [d]compared to the BQ788 + endothelin-1 + citric acid group.

Effect of ET-1

Compared to the saline control group, ET-1 administration caused a significant decrease in Crs (Fig. 1), indicating ET-1-induced airway constriction. However, superimposed ET-1 administration did not, for most cases, significantly augment citric acid-induced airway constriction. The airway constriction induced by the combination of ET-1 and citric acid was significantly attenuated by either BQ123 or BQ788. Using $FEV_{0.1}$ (Fig. 2) and $Vmax_{30}$ (Fig. 3) as indicators, our data also indicate similar changes as those of Crs. The overall trend for the degree of airway constriction was: ET-1 + citric acid = citric acid = ET-1 > BQ123 + ET-1 + citric acid = BQ788 + ET-1 + citric acid = saline control.

Results from Additional Animals

Citric acid aerosol inhalation caused a marked decrease in Crs, indicating severe airway constriction in animals of the citric acid group (Fig. 4). This citric acid-induced airway constriction was significantly attenuated by either BQ123 or BQ788. Results from the other two indicators, $FEV_{0.1}$ and $Vmax_{30}$ (not shown), were similar to those of Crs.

Discussion

We demonstrated citric acid aerosol inhalation- and ET-1-induced airway constriction. The citric acid-induced airway constriction, for most cases, was not significantly augmented by ET-1. However, either BQ123 or BQ 788 significantly attenuated the airway constriction induced by the combination of ET-1 and citric acid. Also, either BQ123 or BQ788 significantly attenuated citric acid-induced airway constriction. Several features of these results will be discussed below.

Citric Acid-Induced Airway Constriction

Inhalation of acidic, including citric acid, acetic acid, and sulfuric acid, aerosols has been shown to induce cough, airway constriction, and increased bronchial responsiveness in patients with asthma (4, 6). Citric acid

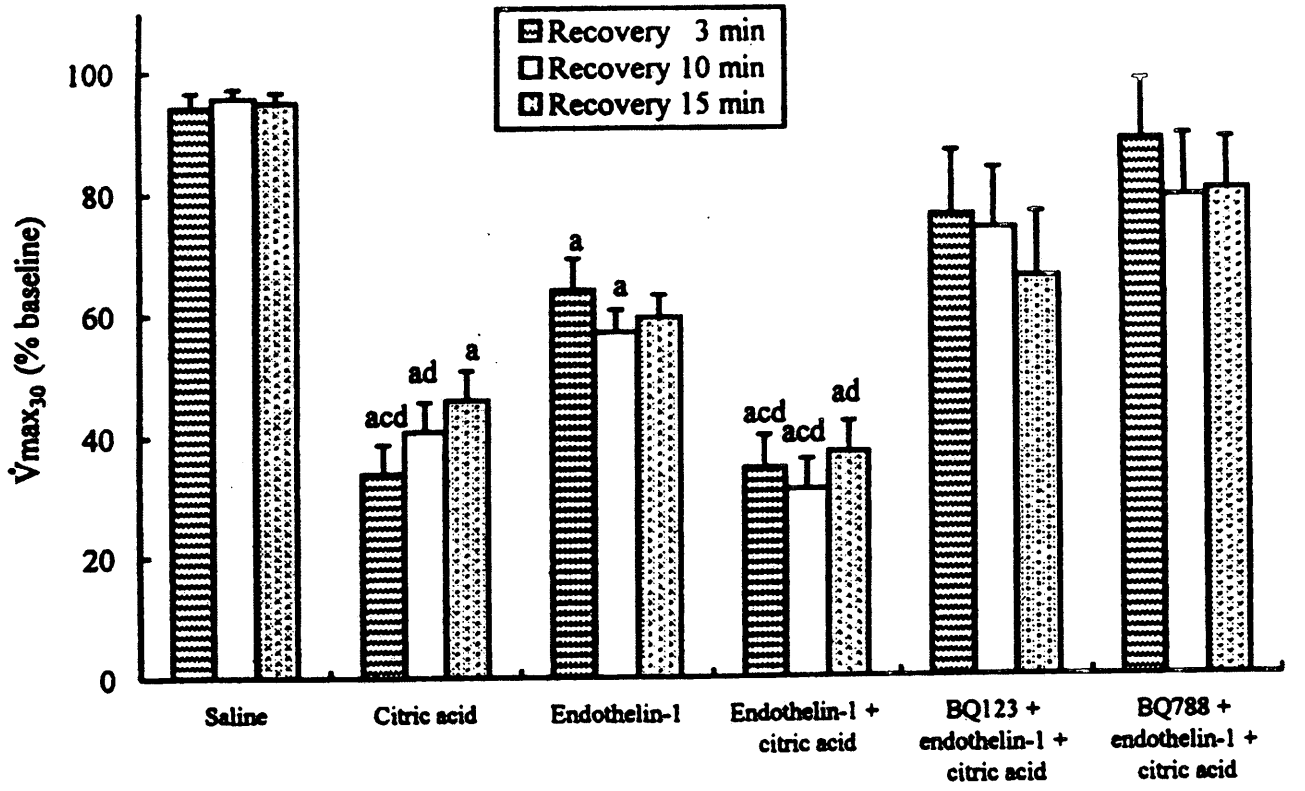

Fig. 3. Citric acid-induced alterations in maximal expiratory flow at 30% total lung capacity ($\dot{V}max_{30}$), expressed as percent baseline values, in six groups of 8 guinea pigs each. Statistical differences ($p < 0.05$) between groups: [a]compared to the saline control group; [b]compared to the endothelin-1 group; [c]compared to the BQ123 + endothelin-1 + citric acid group; [d]compared to the BQ788 + endothelin-1 + citric acid group.

may stimulate the release of tachykinins from the terminals of sensory nerves within bronchial smooth muscle (5, 18). Satoh et al. (24) found that capsaicin pretreatment, capsazepine, or a neurokinin-2 (NK-2) receptor antagonist SR 48968 significantly attenuated citric acid-induced airway constriction. Capsaicin pretreatment depletes tachykinins of afferent C-fibers. Capsazepine is a blocking agent for capsaicin (vanilloid) receptors and may block the effect of citric acid by interfering with proton-sensitive ion channels (1) via occupation of the proposed capsaicin (vanilloid) receptor site (9) of afferent C-fibers. Released tachykinins include substance P (mainly acting on NK-1 receptor), neurokinin A (mainly acting on NK-2 receptor) and neurokinin B (mainly acting on NK-3 receptor). The airway constriction was blocked by SR 48968 (NK-2 receptor antagonist) but not by CP 96486 (NK-1 receptor antagonist), indicating that the constriction is mediated via NK-2, but not NK-1, receptor. Therefore, the findings of Satoh et al. (24) established the relationship between citric acid-induced airway constriction and tachykinins.

In addition, we previously demonstrated that both oxygen radicals and elastase play an important role in tachykinin-mediated, citric acid-induced airway constriction (17). It is possible that both oxygen radicals and elastase enhance the release of tachykinins. Furthermore, Ricciardolo et al. (23) ruled out the potential involvement of another neural component, such as the cholinergic pathway, in citric acid-induced airway constriction in guinea pigs. Yoshihara et al. (27) suggested that citric acid inhalation causes the release of bronchoconstrictor mediators, tachykinins, and simultaneously the release of bronchodilator nitric oxide.

Effect of ET-1 on Citric Acid-Induced Airway Constriction

The bronchopulmonary actions of ET-1 include airway constriction, vasoconstriction and vasodilation. Previous studies have revealed that ET-1 may induce airway constriction either through a direct effect on airway smooth muscle or through an indirect effect secondary to mediator release. ET-1 increases intracellular calcium and activates phospholipase C, and generates inositol triphosphate and diacyl glycerol in human bronchial smooth muscle cells (21). In guinea pig airways, partial inhibition of ET-induced contraction

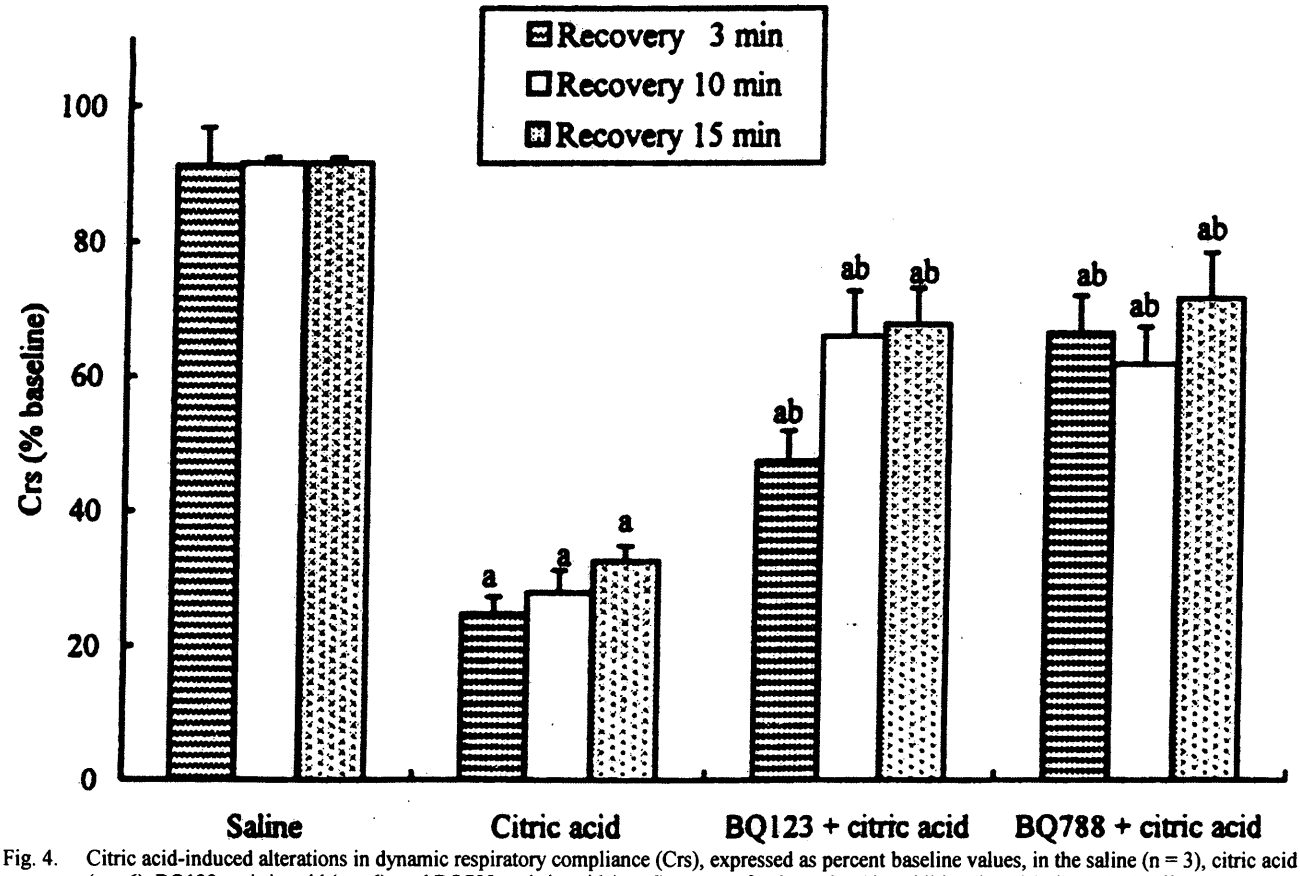

Fig. 4. Citric acid-induced alterations in dynamic respiratory compliance (Crs), expressed as percent baseline values, in the saline (n = 3), citric acid (n = 6), BQ123 + citric acid (n = 6), and BQ788 + citric acid (n = 6) groups of guinea pigs (the additional study). Statistical differences ($p < 0.05$) between groups: [a]compared to the saline control group; [b]compared to the citric acid group.

can be obtained by preincubation with nifedipine (19). For the indirect effect, thromboxane A_2, platelet activating factor and leukotrienes have been suggested as secondary mediators of ET-1-induced airway constriction (7). Furthermore, ET-1 elicits histamine release from guinea pig isolated lung parenchymal mast cells (2).

Kanazawa et al. (11) found that a subthreshold concentration of ET-1 (10^{-10} M) does not enhance the tachykinin release induced by capsaicin but enhances capsaicin-induced airway smooth muscle contraction through ET_B receptors. In addition, ET-1 (10^{-10} M) enhanced exogenous neurokinin A- and substance P-induced airway constriction. It is possible that ET-1 may act through both the direct and the indirect mechanisms, mentioned above, to augment airway constriction caused by capsaicin and tachykinins. Also, ET-1 potentiates cholinergic nerve-mediated contraction in mouse-isolated trachea (13), apparently by activating prejunctional ET_B receptors. This neuronal pathway offers an additional mechanism through which ET-1 may elevate bronchomotor tone. In this study, citric acid-induced airway constriction, for most cases, was not significantly augmented by ET-1 (Figs. 1-3). This lack of augmenting effect was probably due mainly to the fact that we used a high concentration of ET-1 (10^{-8} M) compared to a relatively low concentration of ET-1 (10^{-10} M) in the study of Kanazawa et al. (11).

Both BQ123 and BQ788 significantly attenuated airway constriction induced by the combination of ET-1 and citric acid (Figs. 1-3). These results are compatible with the suggestion that both ET_A and ET_B receptors may be present simultaneously on the same airway smooth muscle cell (12). In addition, both ET_A and ET_B receptors have physiological role in ET-1-induced airway constriction in guinea pigs (22).

Our additional study showed that either BQ123 or BQ788 significantly attenuated airway constriction caused by citric acid alone (Fig. 4). This may indicate that endogenous ET-1 augments citric acid-induced airway constriction, and that either BQ123 or BQ788 significantly attenuated this augmenting effect of endogenous ET-1. This effect of endogenous ET-1 was different from the exogenous ET-1, which did not significantly enhance citric acid-induced airway constriction (Figs. 1-3). It is not clear why there is a

functional difference between the endogenous and exogenous ET-1. It is tempting to explain this difference in the following reason. Since both citric acid and ET-1 caused relatively severe airway constriction, it is easier to demonstrate the attenuating effect than the augmenting effect of the constriction. Thus, in our experimental consequence, especially in the case of the attenuation of citric acid-induced airway constriction by BQ123 and BQ788, it appeared that both ET_A and ET_B receptors are involved in citric acid-induced airway constriction. Nonetheless, neither BQ123 nor BQ788 completely attenuated citric acid-induced airway constriction (Fig. 4). This incomplete effect might also be explained by the direct and the indirect mechanisms as mentioned above. BQ123 and BQ788 could block only the direct, but not the indirect, effect.

In summary, we can infer from our experiment results that ET-1 plays an important role in citric acid aerosol-induced airway constriction in guinea pigs.

Acknowledgements

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