Modification by CO₂ of endothelin-1-induced contraction of isolated guinea pig trachea

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To investigate the effect of changes in CO₂ tension on airway smooth muscle tone induced by various agonists, contractile responses to acetylcholine, histamine and endothelin-1 (ET-1) were studied in isolated guinea pig tracheae at very low PCO₂ (16 ± 0 mmHg, n = 38), moderately low PCO₂ (28 ± 0 mmHg, n = 23), normal PCO₂ (38 ± 1 mmHg, n = 70) or high PCO₂ (94 ± 1 mmHg, n = 32). The minimum concentration of ET-1 (10⁻¹⁰ M) needed to induce contractions was lower than that of acetylcholine (10⁻⁷ M) and histamine (10⁻⁷ M) at normal PCO₂. Changes in PCO₂ did not significantly affect acetylcholine- or histamine-induced contractions. In contrast, very low and moderately low PCO₂ attenuated the contractions induced by ET-1, but high PCO₂ potentiated those induced by a high concentration of ET-1. Very low PCO₂ with normal pH and with high pH attenuated the contractions caused by ET-1, whereas normal PCO₂ with high pH did not. These results suggest that ET-1-induced airway smooth muscle contraction can be modified by PCO₂ per se. Aspirin and indomethacin potentiated the responses to ET-1 at very low PCO₂ more than at normal PCO₂, but attenuated the responses to low concentration of ET-1 at high PCO₂. These results also suggest that cyclooxygenase-related eicosanoids are involved in the effects of PCO₂ on ET-1-induced contractions.

Key words

SMOOTH MUSCLE: tracheal;

GAS: carbon dioxide;

DRUGS: acetylcholine, histamine, endothelin-1;

PROSTAGLANDIN: eicosanoids.

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Ce travail vise à étudier les effets des changements de la tension du CO₂ sur le tonus des muscles lisses respiratoires produits par différents agonistes. La contractilité réactive à l'acétylcholine, l'histamine et l'endothéline-1 (ET-1) est étudiée sur les muscles de trachées isolées de cobaye à des PCO₂ très basses (16 ± 0 mmHg, n = 38), modérées (28 ± 0 mmHg, n = 23) normales (38 ± 1 mmHg, n = 70) ou hautes (94 ± 1 mmHg, n = 32). La concentration minimale de l'ET-1 (10⁻¹⁰) susceptible de déclencher des contractions est plus basse que celle de l'acétylcholine (10⁻⁷) aux PCO₂ normales. Les changements de CO₂ n'affectent pas de façon significative les contractions produites par l'acétylcholine et l'histamine. Au contraire, les PCO₂ très basses ou modérément basses atténuent les contractions provoquées par l'ET-1, mais de hautes PCO₂ potentialisent celles qui sont produites par les hautes concentrations d'ET-1. Des PCO₂ très basses avec pH normal et pH élevé atténuent les contractions provoquées par l'ET-1, alors qu'une PCO₂ normale avec pH élevé ne les change pas. Ces résultats suggèrent que les contractions des muscles lisses de la trachée produites par l'ET-1 peuvent être modifiées par la PCO₂ même. L'aspirine et l'indométhacine potentialisent les effets de l'ET-1 lorsque la PCO₂ est élevée. Ces résultats suggèrent aussi que les eicosanoïdes voisins des cyclooxygénases peuvent être mises en cause pour expliquer les effets de la PCO₂ sur les contractions produites par l'ET-1.

Carbon dioxide is one of major determinants of airway tone and may contribute to the matching of ventilation to perfusion in the lung.^{1,2} Among bronchoconstrictive agonists, it has long been recognized that acetylcholine (ACh) and histamine can regulate airway smooth muscle tone. In fact, in animals and humans cholinergic agonists such as methacholine and carbachol are responsible for the tone of bronchial smooth muscle and thus the parasympathetic nervous system has been considered to have an important role in bronchial asthma.³ In addition, histamine has been considered to be the major inflammatory mediator involved in asthma, since infused histamine causes no bronchoconstriction in normal subjects, whereas it causes bronchoconstriction in asthmatic pa-

tients.⁵ Endothelin-1 (ET-1), a potent vasoconstrictive substance recently isolated from endothelial cells⁵ has been reported to be one of the most potent constrictors of the guinea pig tracheal smooth muscle cells.⁶ Although the possible roles of the substance in physiological and pathological processes remain to be determined, the fact that ET-1 is synthesized by airway epithelial cell^{7,8} suggests that ET-1 could affect adjacent local bronchial tone. The previous studies showed that hypocapnia augments and hypercapnia attenuates contractions of feline and canine airways produced by serotonin and KCl, but not by Ach.⁹⁻¹¹ Since we know no reports of the effects of PCO₂ on ET-1-induced contractions of airway smooth muscle, we studied the contractile responses to ET-1 at various PCO₂ levels in isolated guinea pigs tracheae. It has been reported that membranes permit the free diffusion of carbon dioxide but will not permit the passage of hydrogen ions.¹² Thus, we studied the dependence of the effect of CO₂ on changes in extracellular pH.

Methods

With approval of the Animal Care Committee of Niigata University, guinea pigs (300–500 g) were anaesthetized with intraperitoneal pentobarbital (100 mg · kg⁻¹) and exsanguinated. The tracheae were removed carefully and trimmed of fat and connective tissue. The ring segments (diameter 2.0–2.5 mm, length 3 mm) were isolated and were fixed vertically between hooks at a resting tension of 2.0 g in 20 ml siliconized glass tissue baths containing a modified Krebs solution. The composition of the solution was as follows (mM): Na⁺ 143.0; K⁺ 5.9; Ca⁺⁺ 2.5; Mg⁺⁺ 1.2; Cl⁻ 153.9; HCO₃⁻ 25.0; SO₄²⁻ 1.2; H₂PO₄⁻ 1.2; dextrose 10.0. The solution was maintained at 37° C and was aerated with a mixture of 95% O₂ and 5% CO₂ (pH 7.4). The specimens were connected to a transducer (model TB-612T, Nihon Koden Kogyo Co.) and changes in isometric tension were measured.

After a one-hour equilibration period, the contractions were induced with 80 mM potassium chloride. After repeated washes with fresh Krebs solution, and when the resting tension had stabilized ET-1 (10⁻¹⁰ to 10⁻⁷ M), ACh (10⁻⁷ to 10⁻³ M) or histamine (10⁻⁸ to 10⁻⁴ M) was added to the bath in a cumulative fashion. Only one cumulative concentration-response curve for the agonists was obtained from each preparation. To test the effects of changes in PCO₂, the solutions were aerated with 98.8% O₂ and 1.2% CO₂ (very low PCO₂), 96.8% O₂ and 3.2% CO₂ (moderately low PCO₂), 95.0% O₂ and 5.0% CO₂ (normal PCO₂), and 88.0% O₂ and 12.0% CO₂ (high PCO₂). The CO₂ began flowing ten minutes before the dose-response curves were obtained.

The responses to ET-1 were tested in solutions with

high pH (about 7.8) and normal PCO₂ (about 40 mmHg), and in solutions with normal pH (about 7.4) and very low PCO₂ (about 15 mmHg). To make the solution with high pH and normal PCO₂, the concentration of bicarbonate was increased from 25.0 mM to 68.0 mM. To make the solution with normal pH and very low PCO₂, the concentration of bicarbonate was decreased from 25.0 mM to 10 mM. In both solutions, the concentration of NaCl was adjusted to maintain tonicity.

To determine the role of intrinsic eicosanoids and the effect of PCO₂ on ET-1 contraction, 10⁻⁴ M aspirin or 3 × 10⁻⁶ M indomethacin (cyclooxygenase inhibitors) was added 30 min before exposure to the various concentrations of carbon dioxide. Then the effects of aspirin or indomethacin on the ET-1 dose-response relationship were recorded.

The values presented in the text and figures are means ± standard errors of the means (SEM). The contractions caused by each drug were expressed as percents of those induced by 80 mM potassium chloride. The data were analyzed with one-way analysis of variance followed by the least significant difference test for multiple comparisons. *P* < 0.05 was considered to be statistically significant. The pH and PCO₂ were measured at 37° C with an ABL-3 blood gas analyzer (Radiometer, Copenhagen, Denmark).

Acetylcholine, histamine, and ET-1 were purchased from Daiichi Pharmaceutical Co. (Tokyo, Japan), Aldrich Chemical Co. (Milwaukee, U.S.A.) and Peptide Institute Inc. (Osaka, Japan), respectively. All drugs were dissolved in distilled water except for indomethacin (absolute ethanol) and aspirin (sodium citrate).

Results

The pH and PCO₂ in each group are summarized in the Table. Decreasing the PCO₂ (very low and moderately low PCO₂) increased the pH of the solution to 7.85 and 7.56. Decreasing [HCO₃⁻] of the solution to 10 mM made the pH normal (7.41) despite decreases in PCO₂ (very low PCO₂/normal pH). Adjusting the concentration of bicarbonate to 68.0 mM increased the pH of the solution to 7.81 at normal PCO₂ (Normal PCO₂/high pH).

The lowest concentration required to induce contraction was 10⁻¹⁰ M in ET-1-treated preparations, and 10⁻⁷ M in acetylcholine- and histamine-treated preparations. Contractions caused by 10⁻¹⁰ to 10⁻⁷ M ET-1 were attenuated by very low and moderately low PCO₂, while those caused by 10⁻⁷ M ET-1 were augmented by high PCO₂ (Figure 1). The dose-response curves of acetylcholine and histamine were not influenced by PCO₂ (Figures 2 and 3).

TABLE pH and PCO₂ in each group

Group	n	pH	PCO ₂ mmHg
Very low PCO ₂ (high pH)	38	7.85 ± 0.02	16 ± 0
Moderately low PCO ₂ (high pH)	23	7.56 ± 0.01	28 ± 0
Normal PCO ₂	70	7.40 ± 0.00	38 ± 0
High PCO ₂ (low pH)	32	7.00 ± 0.01	94 ± 1
Very low PCO ₂ (normal pH)	7	7.41 ± 0.01	18 ± 1
Normal PCO ₂ (high pH)	6	7.81 ± 0.01	38 ± 0

Values are means ± SEM. n: number of preparations.

Very low PCO₂ with normal and with high pH attenuated the ET-1-induced contractions. Normal PCO₂ with high pH did not affect the ET-1 dose-response curve. (Figure 4).

Aspirin, 10⁻⁴ M, and indomethacin, 3 × 10⁻⁶ M, decreased the basal tone by 193 ± 36 mg (n = 20) and 24 ± 18 mg (n = 21), respectively. At very low PCO₂, aspirin and indomethacin potentiated the responses to 3 × 10⁻⁸ to 10⁻⁷ M ET-1. The potentiations of the maximal responses to ET-1 by aspirin and indomethacin are 145% and 95%, respectively (Figure 5, left panel). At normal PCO₂, both cyclooxygenase inhibitors potentiated the responses to higher concentrations of ET-1. Aspirin 10⁻⁴ M potentiated the maximal responses to 10⁻⁷ M ET-1 by 44% (Figure 5, centre panel). In contrast, at high PCO₂, 10⁻⁴ M aspirin and 3 × 10⁻⁶ M indomethacin attenuated the responses to low doses of ET-1 (10⁻¹⁰ to 10⁻⁹ M) (Figure 5, right panel).

Discussion

The results of the present study revealed that changes in PCO₂ modified the responses of guinea pig tracheal smooth muscle to ET-1, but not the responses to acetylcholine or histamine. The finding that ACh-induced contractions are insensitive to changes of CO₂ are consistent with previous studies.^{9,10} Although the mechanisms responsible for the differential sensitivity to CO₂ among the agonists is uncertain, the present study showed that the contraction induced by ET-1 of airway smooth muscle cells are very sensitive to changes in PCO₂, especially to low CO₂ tension.

Very low PCO₂ with normal and high pH decreased the responses to ET-1, whereas normal PCO₂ with high pH did not. Therefore, the attenuation of the response to ET-1 was due to low PCO₂ *per se*, and not to the concomitant increase in extracellular pH. It has been reported that intracellular pH affects smooth muscle function in many different smooth muscle cells including airway smooth muscle cells.¹³ Carbon dioxide can easily pass through the cell membranes, but hydrogen ions do

FIGURE 1 Dose-response curves of ET-1 at very low, moderately low, normal and high PCO₂. Numbers of experiments are shown in parentheses. **P* < 0.05, ***P* < 0.01: significantly different from contractions while exposed to normal PCO₂. The contractions induced by ET-1 were attenuated by very low and by moderately low PCO₂, and the contraction induced by 10⁻⁷ M ET-1 was augmented by high PCO₂.

not.¹² In contrast, Duckles *et al.*⁹ reported that in feline bronchial smooth muscle the effect of CO₂ on serotonin-induced contraction was due to a change in extracellular pH. The reason for the discrepancy is unknown but may be related to species differences or to the different agonists used.

In the present study, aspirin and indomethacin potentiated the responses to ET-1 at very low PCO₂ more than at normal PCO₂, but attenuated the responses to low concentration of ET-1 at high PCO₂. This suggests that cyclooxygenase-related eicosanoids are involved in the effects of PCO₂ on these responses and that hypocapnia may release bronchodilating eicosanoids, and hypercapnia bronchoconstricting ones. It has been reported that cyclooxygenase-related metabolites influence airway smooth muscle tone^{14,15} and that ET-1 stimulates the synthesis of prostaglandin E₂ and I₂, and of thromboxane A₂.¹⁶ Prostaglandin E₂ may also be a bronchodilator,^{17,18} and thromboxane A₂ a bronchoconstrictor.¹⁴ Further study is necessary to clarify which eicosanoids are related to the effect of PCO₂ on ET-1-induced airway smooth muscle contraction.

The effect of cyclooxygenase inhibitors on ET-1-induced airway contraction in the guinea pig is contro-

FIGURE 2 Dose-response curves of acetylcholine at very low, moderately low, normal and high PCO₂. Numbers of experiments are shown in parentheses. The contractions induced by acetylcholine were not influenced by PCO₂.

FIGURE 3 Dose-response curves of histamine at very low, moderately low, normal and high PCO₂. Numbers of experiments are shown in parentheses. The contractions induced by histamine were not influenced by PCO₂.

versial. Hay¹⁹ reported that the contraction induced by 3×10^{-8} to 3×10^{-7} M ET-1 was potentiated by indomethacin *in vitro* at normal PCO₂. Maggi *et al.*²⁰ reported inhibition of ET-1-induced contraction by indomethacin at a low PCO₂. In contrast, Schumacher *et al.*²¹ reported that dose-dependent contractions caused by ET-1 *in vitro* were unaffected by indomethacin at normal PCO₂. In the present experiment, aspirin and indomethacin potentiated the ET-1-induced contractions at very low and at normal PCO₂, but attenuated them at high PCO₂. It is not immediately clear why these results differ. However, from our results it appears that cyclooxygenase products may greatly influence the ET-1-induced airway smooth muscle contraction depending on the level of CO₂ tension.

In the present study, the minimum concentration of ET-1 needed to induce tracheal smooth muscle contraction was 10^{-10} M, which is almost 150 times greater than the normal serum concentration of ET-1 ($1.59 \text{ pg} \cdot \text{ml}^{-1}$; 6.4×10^{-13} M).²² However, there is evidence that ET-1 is active locally, rather than systemically,⁸ and most production of ET-1 in the lung occurs in the epithelial cells of bronchioles.^{7,8} Although the local concentration of ET-1 in bronchioles cannot be estimated, our findings suggest that changes in PCO₂ influence bronchomotor tone.

FIGURE 4 The effects of PCO₂ and pH on ET-1-induced contraction. Numbers of experiments are shown in parentheses. *P < 0.05, **P < 0.01: significantly different from contractions while exposed to normal PCO₂ and normal pH. Very low PCO₂ with normal and with high pH attenuated the ET-1-induced contractions. Normal PCO₂ with high pH did not affect the dose-response relationship.

FIGURE 5 Modification by 10^{-4} M aspirin or 3×10^{-6} M indomethacin of the ET-1 dose-response curves at very low (left panel), normal (centre panel) and high (right panel) PCO_2 . Numbers of experiments are shown in parentheses. Aspirin, 10^{-4} M, and indomethacin, 3×10^{-6} M, potentiated the responses to ET-1 at very low PCO_2 more than at normal PCO_2 . In contrast, 10^{-4} M aspirin or 3×10^{-6} M indomethacin attenuated the responses to low concentrations of ET-1 at high PCO_2 .

In summary, changes in PCO_2 modified ET-1-induced contraction in isolated guinea pig tracheae. Cyclooxygenase-related eicosanoids may be involved in the effects of PCO_2 on ET-1-induced contraction.

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