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Augmentation of spontaneous cough by enalapril through up-regulation of bradykinin B_1 receptors in guinea pigs

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Abstract

Studies of angiotensin-converting enzyme inhibitor-induced cough have involved extensive use of experimental models in which guinea pigs are exposed to an inhaled stimulus such as capsaicin or citric acid. In the present study, we examined enalapril-induced potentiation of spontaneous cough in guinea pigs, without an inhaled stimulus. Daily oral administration of enalapril (3 mg/kg) for 20 to 30 days enhanced spontaneous cough. This enhancement of cough was inhibited by the bradykinin B_1 receptor antagonist des-Arg^{10}-[Leu^9]kallidin, but not by the bradykinin B_2 receptor antagonist icatibant. The amount of the bradykinin B_1 receptor agonist [^{3}H]des-Arg^{10}-kallidin specifically bound to membrane fractions from the trachea and larynx was increased by prolongation of the enalapril treatment, and positively correlated well with coughing frequency. In conclusion, the present results indicate that enalapril-induced cough is mediated by up-regulation of bradykinin B_1 receptors.

Keywords: Angiotensin-converting enzyme inhibitor; Bradykinin; Bradykinin B_1 receptor; Bradykinin B_2 receptor; Cough; (Guinea pig)

1. Introduction

Dry cough is the most common side effect of angiotensin-converting enzyme inhibitors (Sesoko and Kaneko, 1985; Morice et al., 1987; Fuller and Choudry, 1987), which are used to treat hypertension and congestive heart failure. Studies of the mechanisms of angiotensin-converting enzyme inhibitor-induced cough have involved extensive use of experimental models using guinea pigs. Long-term daily administration of angiotensin-converting enzyme inhibitors increases the frequency of cough induced by an aerosolized stimulus such as capsaicin or citric acid (Kamei and Kasuya, 1992; Takahama et al., 1993; Ito et al., 1995; Ebihara et al., 1996). In addition, it has been shown that the bradykinin B_2 receptor antagonist icatibant inhibits angiotensin-converting enzyme inhibitor-enhanced cough induced by capsaicin or citric acid (Takahama et al., 1996; Fox et al., 1996), suggesting that kinins are involved in enhancement of induced cough via activation of bradykinin B_2 receptors.

Capsaicin and citric acid can directly activate afferent nerve fibers such as unmyelinated C-fibers in the airway, triggering various pro-inflammatory events (Undem et al., 2002). Therefore, omitting inhaled stimuli such as capsaicin or citric acid should increase precision of analysis of mechanisms underlying angiotensin-converting enzyme inhibitor-induced dry cough in clinical settings.

In the present study, we examined enalapril-induced potentiation of spontaneous cough in guinea pigs. We first evaluated the time-course of induction of cough during daily oral administration of enalapril. Then, we assessed involvement of kinins in the enalapril-induced cough using bradykinin B_1 and B_2 receptor antagonists. Finally, we evaluated whether bradykinin B_1 or B_2 receptor expression in the airway tissue was altered by the daily enalapril treatment, in radioligand-binding experiments using the membrane fraction of the trachea and larynx.

2. Materials and methods

2.1. Animals

Four- to eight-week-old male Hartley guinea pigs (Japan SLC, Hamamatsu, Japan) were used. The animals were housed in an air-conditioned room at 23 ± 1 °C and 60 ± 10% humidity, with lights on from 8:00 AM to 8:00

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PM, for at least 1 week after purchase. They were fed a standard laboratory diet and given water ad libitum.

This animal study was approved by the Experimental Animal Research Committee at Kyoto Pharmaceutical University.

2.2. Reagents

Enalapril maleate, captopril, 1,10-phenanthroline and soybean trypsin inhibitor were obtained from Sigma, St. Louis, MO, USA. Bradykinin diacetate trihydrochloride, des-Arg^{10}-[Leu^{9}]kallidin and icatibant (HOE140) were obtained from Peptide Inst., Osaka, Japan. [2,3-prolyl-3,4-^{3}H(N)]bradykinin ([^{3}H]bradykinin, 3959 GBq/mmol) and [3,4-prolyl-3,4-^{3}H(N)]des-Arg^{10}-kallidin ([^{3}H]des-Arg^{10}-kallidin, 3367 GBq/mmol) were obtained from New England Nuclear, Boston, MA, USA. Aprotinin (Trasylol®) was obtained from Bayer Paham. Ind., Osaka, Japan. Dithiothreitol, bacitracin and bovine serum albumin were obtained from Wako, Osaka, Japan.

2.3. Detection of cough

A conscious animal was placed in a whole-body box without restraint, with ventilation of the inside of the box at a flow rate of 2 l/min. Cough was detected by respiratory pattern and coughing sound. The respiratory pattern was analyzed using a Pulmos-I data analyzer (M.I.P.S., Osaka, Japan) after detection of airflow by a sensor attached to the box. Coughing sounds were detected by a microphone attached inside the chamber, and amplified sounds were heard using an earphone.

2.4. Administration of enalapril

Time-course change of spontaneous cough during 1–30-day treatment of enalapril was evaluated in eight groups, control, and 1-, 5-, 10-, 15-, 20-, 25- and 30-day treatment groups. Control and 30-day treatment groups were daily treated with 2% gum arabic solution (vehicle) and 3 mg/kg enalapril (p.o.), respectively, for 30 days. Treatment groups (1-, 5-, 10-, 15-, 20- and 25-day) were daily treated with 2% gum arabic solution for beginning 29, 25, 20, 15, 10 and 5 days, respectively, and then with 3 mg/kg enalapril for 1, 5, 10, 15, 20 and 25 days, respectively. Measurement of coughing frequency for 30 min was started 2 h after the last administration on day 30.

We decided to administer enalapril at 3 mg/kg 2 h before measurement of coughing frequency because of following findings in our previous study using capsaicin-induced cough of guinea pigs (Hatanaka et al., 1996): (1) When enalapril (10 mg/kg) was administered 2 h before an exposure to capsaicin, the number of capsaicin-induced cough was significantly increased. However, when the enalapril treatment was performed 5 min, 30 min and 1 h before the capsaicin exposure, the cough was not affected. (2) When enalapril was administered at 1, 3, 10 and 30 mg/kg 2 h before the capsaicin treatment, enalapril dose-dependently enhanced the capsaicin-induced cough at 1–10 mg/kg. Enhancing potencies at 3 and 10 mg/kg enalapril were almost equal to each other.

To evaluate time-course changes in coughing frequency after 30-day treatment of enalapril, measurement of coughing frequency for 30 min was started 2, 3 and 4 h after the 30th treatment.

2.5. Effects of bradykinin B_1 and B_2 receptor antagonists

Des-Arg^{10}-[Leu^{9}]kallidin (0.01, 0.1, 1 and 10 nmol/kg) and icatibant (0.01, 0.1, 1 and 10 nmol/kg) were used as bradykinin B_1 and B_2 receptor antagonists, respectively. These compounds were cumulatively administered into veins at the front legs of a conscious guinea pig while the animal was gently held by another investigator. As shown in Fig. 1, at 1 h and 55 min after the 30th administration of enalapril, physiological saline was administered, followed by counting of coughing frequency for 30 min beginning 5 min after the saline injection. Then, 0.01 nmol/kg des-Arg^{10}-[Leu^{9}]kallidin or icatibant was administered, followed by counting of cough frequency for 30 min beginning 5 min after the administration of des-Arg^{10}-[Leu^{9}]kallidin or icatibant. Next, 0.1, 1 and 10 nmol/kg des-Arg^{10}-[Leu^{9}]kallidin or icatibant were cumulatively administered at 35-min intervals, and counting of cough frequency was performed for 30 min beginning 5 min after each administration.

2.6. Radioligand-binding assays of bradykinin B_1 and B_2 receptor agonists

2.6.1. Preparation of crude membrane fraction of the trachea and larynx

In a separate experiment of the time-course study described above, the trachea and larynx of control, and 1-, 10-, 20-, and 30-day treatment groups were isolated following measurement of coughing frequency. The tissues were homogenized (0 °C, 12,000 rpm, 15 s) in 10 mM HEPES buffer (pH 7.4, 9 ml/g wet tissue) containing 250

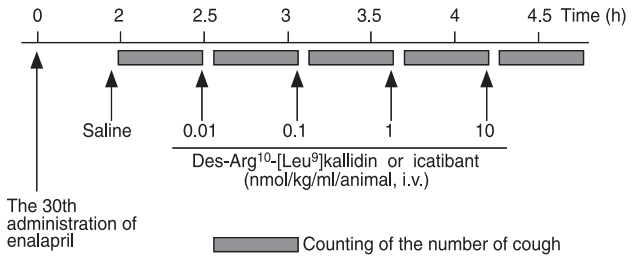

Fig. 1. Schedule for evaluation of effects of bradykinin B_1 and B_2 receptor antagonists on enalapril-induced cough in guinea pigs.

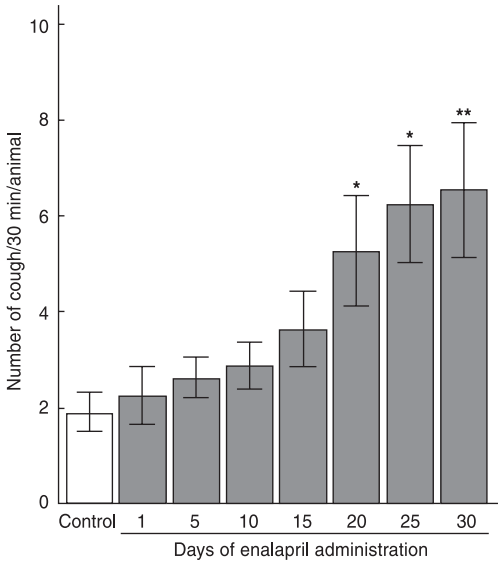

Fig. 2. Daily administration of enalapril-induced cough in guinea pigs. Control and 30-day treatment groups were daily treated with 2% gum arabic solution (vehicle) and 3 mg/kg enalapril (p.o.), respectively, for 30 days. Treatment groups (1-, 5-, 10-, 15-, 20- and 25-day) were daily treated with 2% gum arabic solution for beginning 29, 25, 20, 15, 10 and 5 days, respectively, and then with 3 mg/kg enalapril for 1, 5, 10, 15, 20 and 25 days, respectively. Coughing frequency was counted beginning 2 h after the last administration for 30 min. Each column represents the mean ± S.E.M. of eight animals. *,**Significantly different from the value before administration, at $P<0.05$ and 0.01, respectively.

mM sucrose, 1 mM 1,10-phenanthroline, 140 μg/ml bacitracin, 20 μM captopril, 1 mM dithiothreitol and 15 μg/ml soybean trypsin inhibitor. The homogenate was centrifuged (4 °C, 1000 × *g*, 10 min), and the supernatant was filtered through a nylon mesh (pore size, 100 μm). The filtrate was centrifuged (4 °C, 50,000 × *g*, 10 min), and the pellet obtained was homogenized (0 °C, 12,000 rpm, 15 s) in an assay buffer (25 mM HEPES buffer [pH 7.4] containing 1 mM 1,10-phenanthroline, 140 μg/ml bacitracin, 20 μM captopril and 1 mM dithiothreitol), followed by filtration through a nylon mesh (pore size, 100 μm). Protein concentration in the filtrate was measured by the method of Hartree (1972), who modified the method of Lowry et al. (1951) using bovine serum albumin as a standard. The filtrate was stored at − 80 °C until use.

2.6.2. Binding assays of [^{3}H]des-Arg^{10}-kallidin and [^{3}H]bradykinin

The mixtures used for the binding assays contained 0.5 nM [^{3}H]des-Arg^{10}-kallidin or 0.5 nM [^{3}H]bradykinin, and the membrane fraction of the trachea and larynx (100 μg protein/ml), in the assay buffer, in a final volume of 500 μl.

The specimens were incubated for 60 min at room temperature, and were then vacuum-filtered using glass microfiber filters (GF/C, Whatman, Kent, UK) that had been immersed in 0.2% polyethyleneimine (Sigma). The filters were washed (5 ml × 3 times) with the assay buffer, followed by drying at 80 °C for 15 min, and the radioactivity remaining on each filter was counted using a liquid scintillation counter (LSC-1000, Aloka, Meerbusch, Germany). Specific binding was defined as the difference between total binding and nonspecific binding, determined in the presence of an excess (10 μM) of unlabelled des-Arg^{10}-kallidin or bradykinin.

2.7. Statistical analysis

Statistical analyses were performed using one-way analysis of variance (ANOVA). If significance was detected, individual group differences were evaluated by Bonferroni's multiple test. Correlation was analyzed by Pearson's test. A probability value (P) of <0.05 was considered to indicate statistical significance.

3. Results

3.1. Time-course of effect of enalapril on spontaneous cough

Fig. 2 shows time-course change in coughing frequency induced by daily administration of enalapril for 30 days. Spontaneous cough was detected even in the control group at <2 times/30 min. This spontaneous cough tended to be enhanced by enalapril treatment for 1 to 15 days. In 20-, 25- and 30-day treatment group, enalapril significantly enhanced the spontaneous cough by >5 times/30 min.

As shown in Fig. 3, the cough enhanced by 30-day treatment of enalapril lasted for at least 4.5 h after the 30th enalapril treatment.

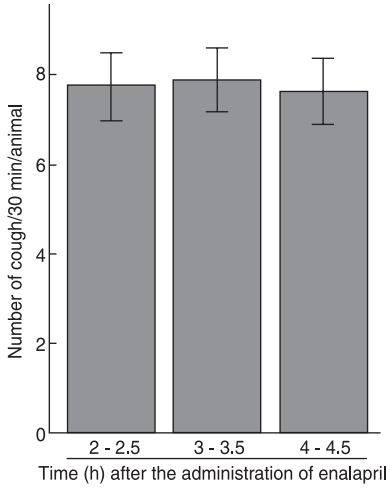

Fig. 3. Time-course change in coughing frequency after the 30th administration of enalapril in guinea pigs. Enalapril was daily p.o. administered at 3 mg/kg/time/day for 30 days. Coughing frequency was counted for 30 min, beginning 2, 3 and 4 h after the 30th administration of enalapril. Each column represents the mean ± S.E.M. of eight animals.

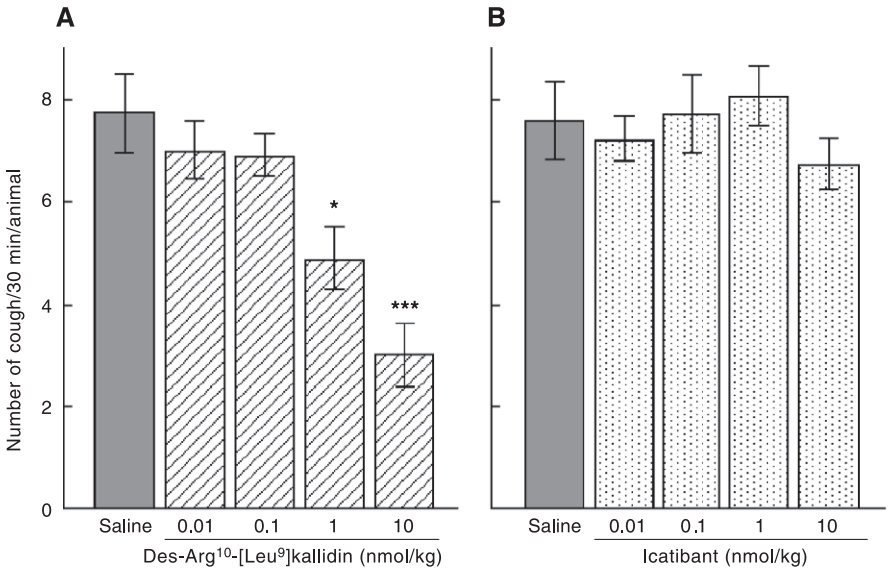

Fig. 4. Effects of bradykinin B_1 receptor antagonist des-Arg^{10}-[Leu^9]kallidin (A) and bradykinin B_2 receptor antagonist icatibant (B) on enalapril-induced cough in guinea pigs. Enalapril was daily p.o. administered at 3 mg/kg/time/day for 30 days. Saline and des-Arg^{10}-[Leu^9]kallidin (0.01–10 nmol/kg) or icatibant (0.01–10 nmol/kg) was cumulatively i.v. administered at 35-min intervals beginning 2 h after the 30th administration of enalapril. Coughing frequency was counted for 5 to 35 min after each administrations of antagonist. The schedule of administration of these antagonists and counting of coughing frequency is shown in Fig. 1 in detail. Each column represents the mean ± S.E.M. of eight animals. *,***Significantly different from the value after saline treatment, at $P < 0.05$ and 0.001, respectively.

3.2. Effects of bradykinin B_1 and B_2 receptor antagonists on the enalapril-induced cough

The fact that the enalapril-induced cough lasted for several hours (Fig. 3) allowed us to administer bradykinin B_1 and B_2 receptor antagonists cumulatively over several hours, as shown in Fig. 1. At 10 nmol/kg, icatibant only slightly affected the cough induced by 30-day administration of enalapril (Fig. 4B). The bradykinin B_1 receptor antagonist des-Arg^{10}-[Leu^9]kallidin significantly inhibited the cough at 1 and 10 nmol/kg, in a dose-dependent manner. The inhibitory rate at 10 nmol/kg des-Arg^{10}-[Leu^9]kallidin was calculated to be approximately 80%, after subtracting the frequency of the control group (approximately 2 times/30 min/animal) from each value (Fig. 4A).

3.3. Time-course changes in amounts of [^{3}H]des-Arg^{10}-kallidin and [^{3}H]bradykinin specifically bound to the membrane fraction of the trachea and larynx during the daily administration of enalapril

Table 1 shows amounts of [^{3}H]des-Arg^{10}-kallidin and [^{3}H]bradykinin specifically bound to the membrane fraction of the trachea and larynx isolated from guinea pigs treated with enalapril for 1 to 30 days. Specific binding of the bradykinin B_1 receptor agonist [^{3}H]des-Arg^{10}-kallidin was detected at very low levels in control animals, but was increased by a single administration of enalapril. Ten to thirty days of enalapril administration further increased specific binding of [^{3}H]des-Arg^{10}-kallidin in proportion to the number of administrations. Specific binding of the bradykinin B_2 receptor agonist [^{3}H]bradykinin to the trachea and larynx of the nontreated animals was decreased by prolongation of the duration of treatment with enalapril; the amount detected in 30-day treatment group was 40% of that of the control.

We analyzed whether changes in amounts of specific binding of [^{3}H]des-Arg^{10}-kallidin and [^{3}H]bradykinin induced by treatment with enalapril for 1 to 30 days correlated with the number of coughs. As shown in Fig. 5A, coughing frequency showed strong positive statistically

Table 1
Amounts of specific binding of [^{3}H]des-Arg^{10}-kallidin and [^{3}H]bradykinin to the membrane fraction of the trachea and larynx isolated from guinea pigs treated with 1- to 30-day administration of enalapril

Period of enalapril treatment	[^{3}H]des-Arg^{10}-kallidin specifically bound (fmol/mg protein)	[^{3}H]bradykinin specifically bound (fmol/mg protein)
Control	0.03 ± 0.51	8.96 ± 2.24
1 day	2.52 ± 0.67	7.31 ± 2.65
10 days	3.11 ± 1.51	7.21 ± 2.56
20 days	4.37 ± 1.40	4.99 ± 2.29
30 days	4.97 ± 1.80	3.84 ± 2.52

Control and 30-day treatment groups were daily treated with 2% gum arabic solution (vehicle) and 3 mg/kg enalapril (p.o.), respectively, for 30 days. Treatment groups (1-, 5-, 10-, 15-, 20- and 25-day) were daily treated with 2% gum arabic solution for beginning 29, 25, 20, 15, 10 and 5 days, respectively, and then with 3 mg/kg enalapril for 1, 5, 10, 15, 20 and 25 days, respectively. The trachea and larynx were isolated together after the last administration. The homogenate of the tissues was used for radioligand-binding assays. Each value represents the mean ± S.E.M. of nine animals.

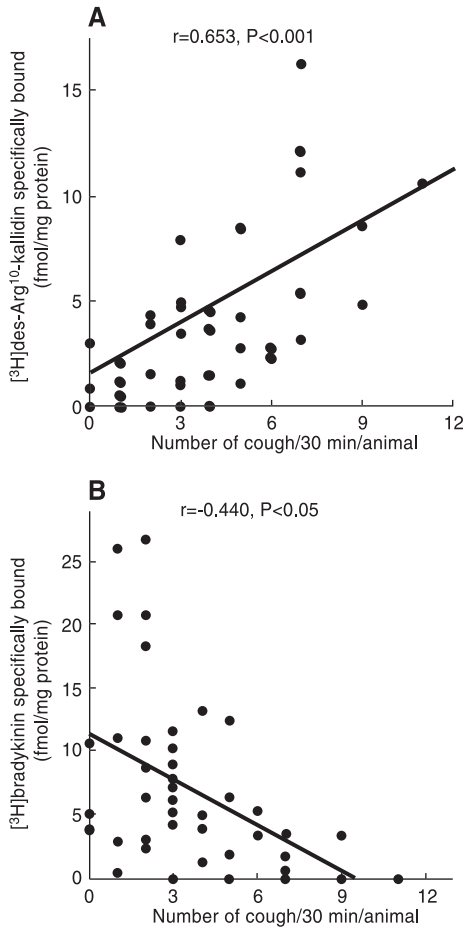

Fig. 5. Correlation between coughing frequency and amount of specific binding of [^{3}H]des-Arg^{10}-kallidin (A) or [^{3}H]bradykinin (B) to the membrane fraction of the trachea and larynx in enalapril-treated guinea pigs.

significant ($r = 0.654$, $P < 0.01$) correlation with the amount of specific binding of [^{3}H]des-Arg^{10}-kallidin. Interestingly, the amount of specific binding of [^{3}H]bradykinin showed negative correlation with the number of coughs ($r = -0.440$, $P < 0.05$) (Fig. 5B).

4. Discussion

There have been no previous reports of whether daily administration of angiotensin-converting enzyme inhibitors induces cough in experimental animals not treated with a stimulus such as capsaicin or citric acid. Inhaled pro-inflammatory stimuli can complicate analysis of mechanisms underlying angiotensin-converting enzyme inhibitor-induced cough. In the present study, we found that treatment with enalapril for more than 20 successive days significantly enhanced spontaneous cough in guinea pigs. This enalapril-induced cough without inhaled stimulus could be a useful model for studies of the mechanisms of angiotensin-converting enzyme inhibitor-induced cough in clinical settings.

Because angiotensin-converting enzyme, also known as kininase II, cleaves endogenous kinins (e.g., the bradykinin B_1 receptor agonists des-Arg^9-bradykinin and des-Arg^{10}-kallidin, and the bradykinin B_2 receptor agonists bradykinin and kallidin) (Brown and Roberts, 2001), administration of enalapril should inhibit degradation of kinins, leading to increased internal kinin concentrations. Indeed, administration of angiotensin-converting enzyme inhibitors has been shown to increase concentration of bradykinin in the serum of humans (Nussberger et al., 1998). In addition, it has been reported that the bradykinin B_2 receptor antagonist icatibant suppressed both capsaicin- and citric acid-induced cough enhanced by angiotensin-converting enzyme inhibitors in guinea pigs (Takahama et al., 1996; Fox et al., 1996), suggesting that these coughs are mediated by activation of bradykinin B_2 receptors, although involvement of bradykinin B_1 receptors was not assessed. We attempted to assess roles of both bradykinin B_1 and B_2 receptor activation in enalapril-induced cough elicited without an inhaled stimulus.

The bradykinin B_1 receptor antagonist des-Arg^{10}-[Leu^9]-kallidin significantly inhibited the cough induced by 30-day administration of enalapril in a dose-dependent fashion. However, interestingly, the bradykinin B_2 receptor antagonist icatibant had no effect on the cough. We confirmed that icatibant effectively suppressed capsaicin-induced cough enhanced by 30-day treatment of enalapril (unpublished data), which is consistent with the results of a study by Takahama et al. (1996). The present results indicate that activation of bradykinin B_1 but not B_2 receptors is largely involved in cough induced by enalapril without capsaicin or citric acid, and that mechanisms of enalapril-enhanced cough in the presence or absence of an inhaled stimulus differ from each other in terms of involvement of bradykinin B_2 receptor activation.

Studies have shown that bradykinin B_2 receptors are constitutively expressed on many cell types (Hall, 1992) including vascular smooth muscle and endothelial cells, and airway epithelial cells (Lung et al., 1998), whereas bradykinin B_1 receptors are expressed at low levels in normal tissue but can be induced in response to pathophysiological stimuli (DeBlois et al., 1991; Marceau et al., 1998). The inhibition of the enalapril-induced cough by the bradykinin B_1 receptor antagonist in the present study suggests that bradykinin B_1 receptors are up-regulated by treatment with enalapril. Thus, in the next step, we attempted to detect amounts of [^{3}H]des-Arg^{10}-kallidin and [^{3}H]bradykinin specifically bound to the membrane fraction of the trachea and larynx that were isolated from enalapril-treated and non-treated guinea pigs.

Only low levels of specific binding of [^{3}H]des-Arg^{10}-kallidin were detected in the tissues of control animals. In enalapril-treated guinea pigs, specific binding of [^{3}H]des-Arg^{10}-kallidin increased in proportion to the prolongation of the treatment period. Furthermore, the amount of specific binding of [^{3}H]des-Arg^{10}-kallidin showed strong positive

correlation with coughing frequency. These results suggest that enalapril-induced cough is closely associated with up-regulation of bradykinin B_1 receptors in airway tissues. We speculate that enalapril increased endogenous levels of kinins, and that pro-inflammatory actions of the accumulated kinins elicited up-regulation of bradykinin B_1 receptors in the airway. To our knowledge, this is the first report to indicate that an angiotensin-converting enzyme inhibitor induces expression of bradykinin B_1 receptors.

In contrast to the results for bradykinin B_1 receptor agonist specific binding, the amount of specific binding of [^{3}H]bradykinin was decreased by prolongation of the duration of enalapril treatment. Similarly, Campos et al. (1996) suggested that systemic treatment with bacterial endotoxin induced up-regulation of bradykinin B_1 receptors in the paw of rats, leading to enhancement of inflammatory responsiveness to a bradykinin B_1 receptor agonist, associated with down-regulation of bradykinin B_2 receptors. In the present study, the reasons why levels of the bradykinin B_2 receptor agonist binding site were decreased by the enalapril treatment are unclear. The amount of specific binding of the bradykinin B_2 receptor agonist showed negative correlation with the number of coughs. These findings are very consistent with the present lack of inhibition of the enalapril-induced cough after icatibant administration, and are further evidence that activation of bradykinin B_2 receptors is not involved in the enalapril-induced cough.

In conclusion, daily administration of enalapril for more than 20 days induced enhancement of spontaneous cough in guinea pigs. The enalapril-induced cough is apparently mediated by activation of bradykinin B_1 receptors, which may be up-regulated by kinins that are likely to be endogenously increased by enalapril.

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