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Studies on the Magnitude and the Mechanism of Cough Potentiation by Angiotensin-Converting Enzyme Inhibitors in Guinea-pigs: Involvement of Bradykinin in the Potentiation

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Abstract

One adverse effect of the angiotensin-converting enzyme (ACE) inhibitors used for treatment of hypertension and congestive heart failure is the production of dry coughs. Imidapril is a new type of ACE inhibitor with a very low incidence of coughs. The magnitude and the mechanism of cough potentiation of imidapril and other ACE inhibitors has been studied in guinea-pigs.

In normal guinea-pigs single and repeated dosing of imidapril at 0·1 to 100 mg kg^{-1} had no effect on capsaicin- or citric acid-induced coughs. Single and repeated dosing of enalapril and captopril at 10 to 30 mg kg^{-1}, respectively, significantly increased the number of capsaicin-induced coughs. Repeated dosing of 1 mg kg^{-1} enalapril also significantly augmented the capsaicin cough. In bronchitic guinea-pigs imidapril also had no effect on the coughs induced by the two stimulants. Enalapril and captopril significantly increased the number of coughs induced not only by capsaicin but also by citric acid. Lower doses of enalapril were enough to augment the capsaicin-induced coughs, whereas medium to large doses failed to augment the cough irrespective of the protocol of administration. Bradykinin-induced discharges of the vagal afferents from the lower airway were significantly increased by enalaprilat but not by imidaprilat. Capsaicin-induced discharges of the afferents were, on the other hand, significantly depressed by enalaprilat, but not by imidaprilat. Interestingly, enalaprilat depression of the discharges was significantly reversed by Hoe-140, a bradykinin B_2 receptor blocker. In guinea-pigs pretreated with a low dose of enalapril, arterial infusion of bradykinin significantly potentiated the coughs induced by capsaicin.

The results indicated that imidapril was less potent than enalapril and captopril in potentiating cough responses induced by capsaicin and citric acid in guinea-pigs, and further suggest that bradykinin might be a key substance in the mechanism of the potentiation of coughs associated with ACE inhibitors.

Angiotensin-converting enzyme (ACE) inhibitors, widely used for treatment of hypertension and congestive heart failure, have been increasingly developed for the past decade. Although in this period, improvements have been made to reduce an incidence of adverse effects such as taste disturbance and dry cough, all ACE inhibitors developed so far seem to be still associated with cough production, which is reversible when the drug is discontinued. The incidence of cough production varies between 1 and 15%, depending on the ACE inhibitor (Coulter & Edwards 1987; Hood et al 1987).

Although we have previously reported that ACE inhibitors had different modes of cough augmentation in guinea-pigs (Takahama et al 1993), it is unclear whether or not cough production is associated essentially with ACE inhibitors. The mechanism of cough production associated with consecutive dosing of ACE inhibitor is also still controversial.

Attention has recently been focussed on imidapril as a new type of ACE inhibitor with a very low incidence of coughs in clinical trials (Sasaguri et al 1994). The present study was designed firstly to determine the magnitude and mode of cough potentiation associated with imidapril in guinea-pigs, in comparison with those associated with enalapril and captopril. Secondarily, the influence of imidapril and enalapril on airway vagal afferent discharges in guinea-pigs was studied to help to elucidate the mechanism of cough potentiation by ACE inhibitors in guinea-pigs, addressing the role of bradykinin in the augmentation. Finally, the cough augmenting action of bradykinin, administered by close arterial injection to the lower airway, was verified in guinea-pigs.

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Materials and Methods

Animals

Male Hartley strain guinea-pigs, 5–7 weeks old, were used in all experiments. Animals used in cough potentiation experiments were housed at room temperature 24 ± 2°C, humidity 55 ± 15%, ventilation 15 times h^{-1}, with a 12-h light-dark cycle. Bronchitis was induced in guinea-pigs by exposure to 200 ppm SO_2 gas for 2 h a day for 7 days using the apparatus (ML-10, Medical Agent) described previously (Kase et al 1982). The guinea-pigs exposed to SO_2 under these conditions showed bronchitis characterized by an increase in the number of inflamed cells in fluid from broncho-alveolar lavage, an increase in the sensitivity of the bronchial smooth muscle to acetylcholine and histamine, and a reduction of neutral endopeptidase (NEP) activity in the trachea (Fuchikami 1992). A few of the animals used sometimes coughed during SO_2 exposure but did not cough spontaneously after termination of the exposure period. To facilitate detection of cough poten-

tiation by ACE inhibitors, the normal and bronchitic animals selected as experimental animals were those in which the number of coughs induced by cough stimulants was 1–4 in 15 min. This number of cough responses was used as the preadministration value of the response. A group consisting of 6 animals was used for each dose of drug. The animals were fasted for 24 h in single dosing experiments, and for 14 h in chronic dosing experiments, but had free access to drinking water.

Drugs

The drugs used were imidapril, enalapril, captopril, imidaprilat, enalaprilat, SR-48968 (gifts from Tanabe Seiyaku, Japan); capsaicin (Sigma); bradykinin, D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin (Peptide Inst., Japan); Hoe-140 (gift from Hoechst Japan); CP-96345 and CP-99994 (gifts from Pfizer); and citric acid (Nakarai tesq, Japan). D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin and Hoe-140 are antagonists of the bradykinin B_2 receptor, CP-96345 and CP-99994 are antagonists of the NK_1 receptor, and SR-48968 is an antagonist of the NK_2 receptor. ACE inhibitors were suspended in 0·5% carmellose sodium solution. Imidaprilat was dissolved into 0·5 M $NaHCO_3$ and then diluted in saline. Enalaprilat and bradykinin were dissolved in saline. Capsaicin was dissolved in 10% ethanol and 10% Tween 80 and then diluted with saline before use. Citric acid was dissolved in distilled water. Pharmacological blockers were dissolved into dimethylsulphoxide. Drugs given into the artery were finally diluted in Tyrode's solution (pH 7·4).

Experiments on cough responses

Non-anaesthetized animals were placed individually in a double-flow plethysmograph (PUL10M, MIPS) and forced to inhale 0·1 M (or 0·03 M) citric acid or 10^{-8} M (or 10^{-9} M) capsaicin solution for 2 min using an ultrasonic nebulizer (NEU12, Omron), connected to the chamber and adjusted to 0·6 mL min^{-1} of the output volume. Coughing was monitored and recorded on a recorder through a pressure transducer (TA240, Gould Electronics) as changes in pressure in the plethysmograph. The sound of coughing was also recorded on a tape recorder means of a small microphone placed within the plethysmograph. The animals were continuously observed by a trained and uninformed observer during the experiment. Coughing could be easily distinguished from sneezing, because there was a clear difference between the two in sound as well as in the behaviour of the animals. The number of coughs during a 2-min inhalation period and the subsequent 13 min was counted. Each ACE inhibitor was given orally 2 h before inhalation of cough inducers.

In the multiple-dose experiment, cough production was confirmed before the first dose, as described above, and the number of coughs was taken as the pre-administration control value. Dosing was performed for 8 days. Two hours after the final dose, the animals were again tested for cough production. In antagonistic studies, guinea-pigs were treated with oral enalapril at 30 mg kg^{-1} 2 h before inhalation of 10^{-8} M capsaicin. Each blocker was given intravenously 3 min before capsaicin challenge. The blockers used were: D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin, Hoe-140, CP-96345, CP-99994, and SR-48968. In experiments on the cough-potentiating effect of bradykinin, bradykinin was infused through the brachial artery to the lower airway for 15 min by means of an infusion pump (1235, Atom). Two-min inhalation with 10^{-8} M capsaicin started at the time of initiation of infusion. The number of coughs was counted during the period of infusion. In all the experiments on cough responses, the effect was evaluated as an increase or decrease in the number of coughs; the number of coughs before and after drug administration was statistically compared initially by using the Kruskal–Wallis assay and, if necessary, further by Dunnett's multiple test. The effect was considered as statistically significant when $P < 0.05$.

Experiments on airway vagal afferent discharges

Guinea-pigs were anaesthetized with urethane (1·5 g kg^{-1}, i.p.). The fine nerve fascicle of approximately 50 μm in diameter which responded to inhaled citric acid solution was detached from the right vagus. The nerve fascicle was drawn on to a small vinyl sheet containing 40 μL of Tyrode's solution in which voltage could be recorded using a platinum-iridium filament electrode 120 μm in diameter. The discharges were conventionally processed through a biophysical amplifier (AVB-10, Nihon kohden), monitored on an oscilloscope (VC-10, Nihon kohden) and recorded on a data-recorder (MR-10, TEAC) for further analysis. For quantitative analysis of the data, the discharges before and after drug administration were integrated into 1-s periods by an integration amplifier (EI-601G, Nihon kohden). Bradykinin or capsaicin was administered by close arterial injection into the lower airway in a volume of 10 μL/100 g body weight, and an active form of ACE inhibitor given into the vein 5 min before stimulant administration. The integrated value of bradykinin- or capsaicin-induced discharges before ACE inhibitor administration was taken as 100%. ACE inhibitor-induced percentage changes in the discharges were statistically compared with that of vehicle control group by using Student's unpaired *t*-test. $P < 0.05$ was considered significant.

Ethical approval

This study was approved by the Committee of the Animal Welfare and Control of Faculty of Pharmaceutical Sciences, Kumamoto University.

Results

Cough-potentiation study in normal guinea-pigs

Numerical data are shown in Table 1. Single doses of ACE inhibitors did not increase the cough response induced by citric acid. The number of capsaicin-induced coughs was significantly increased by 10 and 30 mg kg^{-1} enalapril but not by other ACE inhibitors. Chronic dosing with ACE inhibitors had little effect on citric acid-induced coughs, but chronic doses of captopril significantly increased the number of coughs caused by capsaicin, confirming our previous finding (Takahama et al 1993). Enalapril in chronic doses of 10 and 30 mg kg^{-1} did not potentiate capsaicin-induced coughs, although single administration of the same doses potentiated the coughs. A lower dose of enalapril did, however, significantly potentiate the cough caused by capsaicin.

Cough potentiation study in bronchitic guinea-pigs

The data are summarized in Table 2. Single and chronic dosing of imidapril had no effect on the coughs induced by citric acid or capsaicin in bronchitic or normal guinea-pigs. In single

Table 1. Effects of single and chronic administration of ACE-inhibitors on coughing induced by citric acid and capsaicin in normal guinea-pigs.

ACE inhibitor	Dose (mg kg^{-1})	Single				Chronic			
		Citric acid		Capsaicin		Citric acid		Capsaicin	
		Before	2 h	Before	2 h	Before	8 days	Before	8 days
Control	–	2·0 ± 0·4	1·8 ± 0·3	1·7 ± 0·3	1·7 ± 0·6	1·5 ± 0·2	1·3 ± 0·6	2·0 ± 0·3	2·3 ± 0·6
Imidapril	0·1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2·2 ± 0·4	2·5 ± 1·2
	0·3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2·2 ± 0·5	1·7 ± 0·4
	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2·2 ± 0·4	2·2 ± 0·5
	10	2·2 ± 0·5	0·8 ± 0·5	1·7 ± 0·5	1·5 ± 0·6	1·5 ± 0·3	1·2 ± 0·5	1·8 ± 0·4	1·2 ± 0·4
	30	2·2 ± 0·5	0·3 ± 0·2	1·7 ± 0·3	1·2 ± 0·5	1·5 ± 0·2	1·0 ± 0·4	1·7 ± 0·3	1·3 ± 0·5
	100	1·5 ± 0·2	0·8 ± 0·2	1·5 ± 0·2	2·0 ± 0·5	1·3 ± 0·2	1·7 ± 0·6	1·7 ± 0·3	2·0 ± 0·4
Enalapril	0·1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2·0 ± 0·4	3·5 ± 0·9
	0·3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1·8 ± 0·3	3·2 ± 0·5
	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2·2 ± 0·5	7·8 ± 2·1*
	3	2·2 ± 0·5	4·3 ± 1·2	1·5 ± 0·2	1·5 ± 0·5	1·7 ± 0·5	1·3 ± 0·8	1·7 ± 0·3	1·8 ± 0·4
	10	2·2 ± 0·5	0·8 ± 0·5	2·2 ± 0·5	6·7 ± 0·6**	1·5 ± 0·3	1·7 ± 0·5	1·8 ± 0·3	1·8 ± 0·5
	30	1·5 ± 0·3	0·8 ± 0·3	2·2 ± 0·5	6·5 ± 0·4**	1·5 ± 0·3	4·7 ± 1·2	1·5 ± 0·3	2·3 ± 0·5
Captopril	3	1·7 ± 0·2	1·7 ± 0·6	1·5 ± 0·3	1·7 ± 0·6	1·5 ± 0·3	2·0 ± 0·6	1·7 ± 0·3	2·7 ± 0·6
	10	1·8 ± 0·3	1·5 ± 0·5	1·8 ± 0·3	0·7 ± 0·4	1·5 ± 0·3	1·2 ± 0·6	1·8 ± 0·3	6·0 ± 0·9**
	30	1·8 ± 0·3	1·8 ± 0·6	2·2 ± 0·5	3·8 ± 0·6	1·3 ± 0·2	3·8 ± 0·9	1·8 ± 0·5	5·8 ± 0·6**

Each datum, expressed as the mean ± s.e. of six experiments, represents the number of coughs in 15 min. n.d. not determined; *$P < 0.05$, **$P < 0.01$ compared with control.

doses enalapril and captopril significantly increased the number of coughs induced by citric acid. Single dosing with captopril also potentiated capsaicin-induced coughs. Enalapril potentiation of capsaicin-induced coughs did not occur even after a single dose of 30 mg kg^{-1}, which potentiated citric acid-induced coughs. Lower dosing of all ACE inhibitors did not, however, potentiate citric acid- and capsaicin-induced coughs, except that one dose of enalapril (0·3 mg kg^{-1}) and captopril (1 mg kg^{-1}) potentiated citric acid- and capsaicin-induced coughs, respectively.

Effect on pharmacological blockers on cough potentiation due to enalapril

This study was performed on normal guinea-pigs. Enalapril at 30 mg kg^{-1} increased the number of coughs induced by capsaicin from 1·5 ± 0·3 to 7·2 ± 1·0. As shown in Fig. 1,

Table 2. Effects of single and chronic administration of ACE inhibitors on coughing induced by citric acid and capsaicin in bronchitic guinea-pigs.

ACE inhibitor	Dose (mg kg^{-1})	Single				Chronic			
		Citric acid		Capsaicin		Citric acid		Capsaicin	
		Before	2 h	Before	2 h	Before	8 days	Before	8 days
Control	–	2·0 ± 0·4	1·8 ± 0·3	1·2 ± 0·2	1·3 ± 0·2	1·5 ± 0·3	2·5 ± 0·6	1·3 ± 0·2	3·0 ± 0·9
Imidapril	0·1	n.d.	n.d.	1·8 ± 0·3	1·7 ± 0·4	2·0 ± 0·4	2·0 ± 0·6	1·8 ± 0·5	2·0 ± 0·8
	0·3	n.d.	n.d.	1·8 ± 0·4	2·2 ± 0·6	2·0 ± 0·4	2·8 ± 0·9	2·3 ± 0·6	4·0 ± 0·8
	1	n.d.	n.d.	1·8 ± 0·3	1·0 ± 0·3	2·0 ± 0·4	2·2 ± 0·6	1·8 ± 0·4	1·8 ± 0·9
	10	2·2 ± 0·5	1·8 ± 0·3	2·3 ± 0·5	1·3 ± 0·3	2·0 ± 0·4	2·0 ± 1·1	1·3 ± 0·2	3·3 ± 1·0
	30	1·0 ± 0·0	1·3 ± 0·4	1·3 ± 0·3	2·5 ± 0·9	1·5 ± 0·3	3·0 ± 1·3	1·3 ± 0·2	3·2 ± 0·7
	100	1·0 ± 0·0	4·5 ± 1·1	1·8 ± 0·5	3·3 ± 0·7	1·5 ± 0·2	2·5 ± 1·0	1·5 ± 0·3	2·8 ± 0·9
Enalapril	0·1	n.d.	n.d.	1·5 ± 0·2	3·3 ± 1·9	2·2 ± 0·5	1·5 ± 0·6	2·3 ± 0·5	3·3 ± 0·7
	0·3	n.d.	n.d.	1·7 ± 0·5	5·2 ± 0·9**	2·2 ± 0·5	7·7 ± 1·8*	2·3 ± 0·6	4·2 ± 1·6
	1	n.d.	n.d.	1·5 ± 0·3	8·2 ± 2·4**	2·5 ± 0·4	5·7 ± 1·5	2·3 ± 0·5	6·7 ± 0·7*
	3	2·2 ± 0·5	1·7 ± 0·6	1·7 ± 0·5	2·8 ± 0·8	1·7 ± 0·3	3·7 ± 0·9	1·3 ± 0·2	5·8 ± 1·8
	10	2·2 ± 0·5	4·7 ± 1·3	1·2 ± 0·2	3·3 ± 1·2	1·5 ± 0·3	5·5 ± 1·5	1·3 ± 0·2	3·3 ± 1·2
	30	1·0 ± 0·0	9·5 ± 1·5**	1·5 ± 0·2	3·7 ± 0·9	1·5 ± 0·2	6·5 ± 1·5	1·3 ± 0·2	4·8 ± 0·9
Captopril	3	2·0 ± 0·4	3·8 ± 1·2	2·3 ± 0·4	2·7 ± 0·7	1·5 ± 0·2	3·0 ± 1·2	1·3 ± 0·2	3·2 ± 0·6
	10	1·7 ± 0·4	6·0 ± 1·3*	1·2 ± 0·2	10·0 ± 2·1**	1·3 ± 0·2	5·8 ± 0·9	1·5 ± 0·3	5·5 ± 0·8
	30	1·2 ± 0·2	7·8 ± 2·4*	1·3 ± 0·2	9·7 ± 2·7**	1·7 ± 0·3	7·0 ± 2·1	1·5 ± 0·3	5·7 ± 1·5

Each datum, expressed as the mean ± s.e. of six experiments, represents the number of coughs in 15 min. n.d. not determined; *$P < 0.05$, **$P < 0.01$ compared with control.

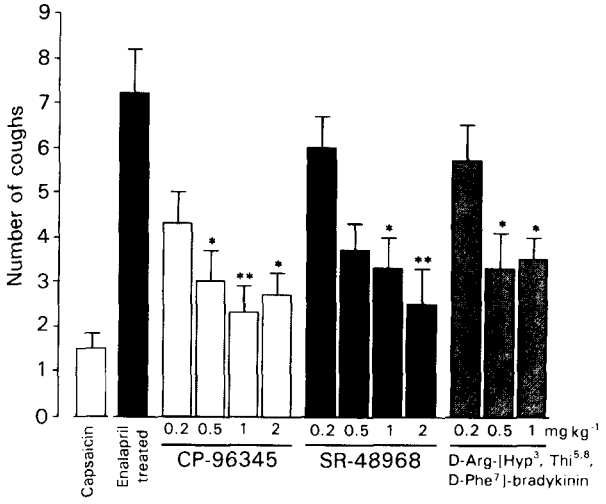

FIG. 1. Effects of CP-96345, SR-48968 and D-Arg-[Hyp^3, Thi^{5,8},D-Phe^7]-bradykinin on augmentation of capsaicin-induced cough responses by enalapril. Enalaparil at 30 mg kg^{-1} was administered 2 h before capsaicin inhalation. Each blocker was given intravenously 3 min before capsaicin challenge. $*P < 0.05$, $**P < 0.01$ compared with control.

coughs potentiated by enalapril were significantly depressed by CP-96345, an NK_1 receptor blocker, SR-48968, an NK_2 receptor blocker and D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin, a bradykinin B_2 receptor blocker, although none of the blockers completely depressed the potentiation.

Effect on bradykinin-induced discharges

Bradykinin in the dose range of 0·1–10 nmol kg^{-1} dose-dependently increased the afferent vagal discharges. Bradykinin-induced discharges were completely depressed by Hoe-

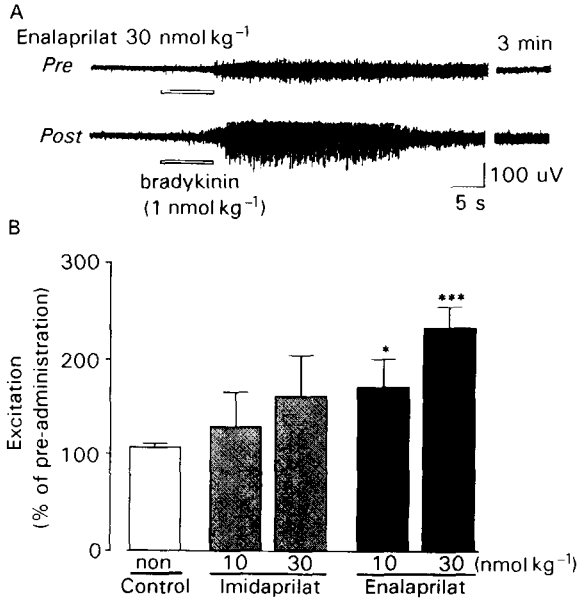

FIG. 2. Effects of imidaprilat and enalaprilat on bradykinin-induced discharges in airway vagal afferents. A. Typical recording of potentiating effect of enalaprilat on the discharges. B. Summarized data of the effects on the bradykinin-induced discharges; Pre, pre-administration; Post, post-administration. Each value is the mean ± s.e. of 4 or 5 experiments. $*P < 0.05$, $***P < 0.001$ compared with control.

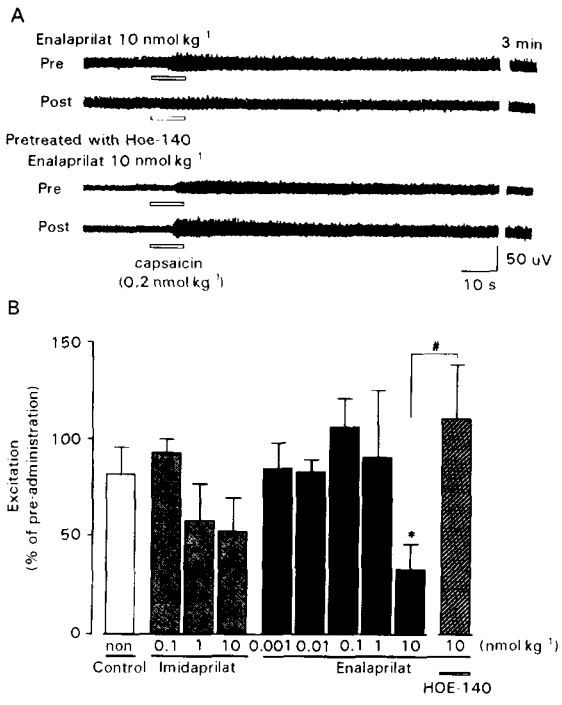

FIG. 3. Effects of imidaprilat and enalaprilat on capsaicin-induced discharges in airway afferent discharges. A. Typical recording of the effect of enalaprilat on capsaicin-induced discharges, and reversal of enalaprilat depression by Hoe-140. Enalaprilat at 10 nmol kg^{-1} was administered 5 min before capsaicin administration. Note that capsaicin discharges, unlike bradykinin discharges, disappeared on administration of enalaprilat. Hoe-140 at 2 nmol kg^{-1} was administered by close arterial injection to the lower airway, 10 min before capsaicin administration. B. Summarized data of the effect on the capsaicin-induced discharges. Note that enalaprilat depression of the discharges was reversed by pretreatment with Hoe-140. Pre, pre-administration; Post, post-administration. Each value is the mean ± s.e. of 4 or 5 experiments. $*P < 0.05$ compared with control $\#P < 0.01$.

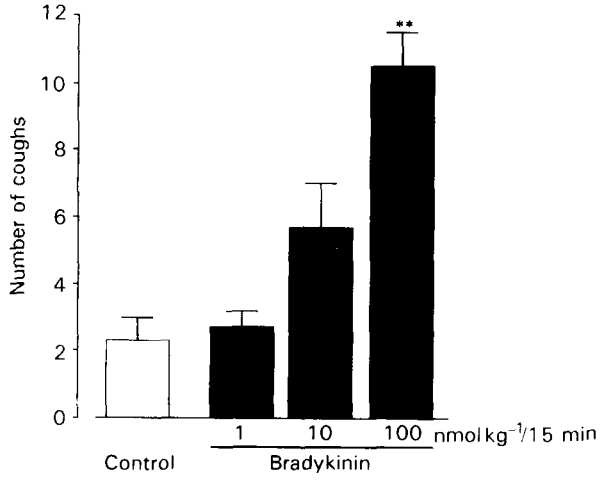

FIG. 4. Effect of intra-arterial infusion of bradykinin on capsaicin-induced cough responses. Two-minutes inhalation of 10^{-8} M capsaicin started at the time of initiation of bradykinin infusion. The number of coughs was counted for 15 min of infusion. $**P < 0.01$ compared with control.

140, a bradykinin B_2 receptor antagonist, and also by CP-99994, an NK_1 receptor antagonist (data not shown). Enalaprilat, an active metabolite of enalapril, at 10 and 30 nmol kg^{-1} significantly potentiated the discharges induced by 1 nmol kg^{-1} bradykinin (Fig. 2B). A representative record of enalaprilat potentiation is shown in Fig. 2A. The same dose of imidaprilat, an active metabolite of imidapril, did not, on the other hand, significantly increase the discharges (Fig. 2B).

Effect on capsaicin-induced discharges

Although capsaicin in doses of 0·02–2·0 nmol kg^{-1} also increased afferent vagal discharges, the discharges were reduced by repeated administration of doses greater than 0·3 nmol kg^{-1}, suggesting that capsaicin responses might be desensitized. Because reproducible responses were obtained to repeated administration of 0·2 nmol kg^{-1} capsaicin at 30-min intervals, this dose- and time-schedule was employed in the experiment.

Capsaicin-induced discharges were dose-dependently depressed by close arterial injection of enalaprilat. A typical recording of the capsaicin depression is shown in Fig. 3A. The effect of 10 nmol kg^{-1} enalaprilat was statistically significant. Interestingly, this depression was significantly inhibited by pretreatment with 2 nmol kg^{-1} of Hoe-140 (Fig. 3). Imidaprilat at 0·1 to 10 nmol kg^{-1} did not, on the other hand, significantly reduce responses to capsaicin (Fig. 3B).

Cough potentiating effect of bradykinin

Bradykinin, when infused alone at 1–10 nmol kg^{-1} for 15 min, did not potentiate coughs caused by capsaicin. Even at a large dose of 100 nmol kg^{-1}, bradykinin alone failed to potentiate capsaicin coughs significantly, although the number of coughs increased 6·3-fold. In guinea-pigs pretreated with 0·1 mg kg^{-1} enalapril, which did not potentiate capsaicin- and citric acid-induced coughs, 1, 10 and 100 nmol kg^{-1} of bradykinin increased the number of the coughs by 2·7, 5·7 and 10·5 times on average, respectively. The effect of 100 nmol kg^{-1} was statistically significant (Fig. 4).

Discussion

This study revealed that imidapril, unlike enalapril and captopril, did not potentiate the cough responses caused by various conditions. It is unlikely that the difference in the potentiation can be attributed to the bioavailability of each ACE inhibitor, because the doses used are comparable with plasma concentrations of active metabolites of ACE inhibitors (Kawashima et al 1994). The difference is, furthermore, not a result of the difference between the ACE-inhibiting activities of enalapril and imidapril, because in the doses used both inhibitors showed the same magnitude of depressor effect on angiotensin I-induced pressor responses in guinea-pigs (unpublished data), and because in ACE-inhibiting activities, assayed by using the trachea, lung, aorta and plasma of guinea-pigs (unpublished data), enalapril was one third as active as imidapril, indicating both active metabolites are comparable in ACE-inhibiting activity.

In normal guinea-pigs, enalapril and captopril potentiated the coughs induced by capsaicin but not by citric acid. Capsaicin releases substance P from the afferent C-fibre of the airway (Saria et al 1988) to stimulate coughing (Forsberg & Karlsson 1986). Citric acid appears, however, to stimulate mainly the A-fibre terminal to produce coughing, as inhaled local anaesthetics, which paralyse the C-fibre, more strongly depressed the coughs caused by capsaicin than those caused by citric acid (Choudry et al 1990). Thus, it is likely that the C-fibre is the major fibre involved in the cough potentiation in normal guinea-pigs.

A single 10 to 30 mg kg^{-1} dose of enalapril potentiated capsaicin- and citric acid-induced cough responses in normal and bronchitic guinea-pigs, respectively, but chronic administration of the same doses resulted in no potentiation. Captopril, on the other hand, potentiated capsaicin-induced coughing in normal animals after chronic dosing but not after single dosing. It seems unlikely that the difference in cough potentiation between the two types of dosing come from the bioavailability of each ACE inhibitor, as there was no significant difference between the maximum concentration, C_{max}, the time at which this occurred, t_{max}, and the half-life, $t_{½}$, for single and chronic dosing of enalapril (Nakashima et al 1984). It has, furthermore, been reported that 10 mg kg^{-1} enalapril produced a progressive fall in blood pressure with a peak on the 7th day of chronic dosing (Oomura et al 1985). The same dose of captopril, on the other hand, produced the maximum hypotensive effect on the 4th day of chronic dosing and the effect gradually decreased despite further dosing (Oomura et al 1985). Our preliminary study revealed that 10 mg kg^{-1} enalapril increased the level of angiotensin I in the lung of guinea-pigs compared with that resulting from administration of 10 mg kg^{-1} captopril. Because ACE converts angiotensin I to angiotensin II (Cushman & Cheung 1971) and also degrades bradykinin (Yang et al 1970) and substance P (Cascieri et al 1984; Yokosawa et al 1985; Skidgel & Erdos 1987), it seems possible that chronic dosing of enalapril caused desensitization of the afferent fibre of the cough reflex to cough inducers such as substance P and bradykinin. The result of chronic dosing of captopril needs further study to clarify the mechanism.

A neutral endopeptidase (NEP) degrades not only tachykinins but also bradykinin (Ichinose & Barnes 1990). Because, in bronchitic guinea-pigs, the NEP activities in the trachea and bronchus were reduced (Fuchikami 1992), the level of bradykinin and tachykinins in the airways should be increased in such animals. The potentiating effect of 3–10 mg kg^{-1} enalapril on capsaicin-induced coughs did not, however, occur in bronchitic guinea-pigs. The threshold of cough responses to capsaicin stimulation is reduced in bronchitic animals (Fuchikami et al 1990; Fuchikami 1992; Sakata et al 1993). This fact was preliminarily confirmed in the present study. Thus, enalapril might have facilitated the desensitization of cough response caused by capsaicin in bronchitic animals. This contention should be supported by the result that even in bronchitic guinea-pigs low doses of enalapril potentiated the coughs caused by capsaicin. This seems to be adapted to the effects of captopril on capsaicin-induced coughs in bronchitic animals.

Before electrophysiological studies, we performed several basic experiments to confirm that the discharges are a consequence of excitation of the vagal afferents arising from the broncho-alveolar regions (data not shown): inhalation of citric acid or capsaicin solution caused increased discharges of the afferents used; basal discharges increased in bronchitic guinea-

pigs, compared with those in normal guinea-pigs; and capsaicin- and bradykinin-induced discharges did not change after ablation of the abdominal vagal nerves. Because quantitative analysis of the changes in the discharges was difficult when stimulants were given by inhalation, stimulants were administered by close arterial injection to the broncho-alveolar regions. It is, furthermore, likely that capsaicin- and bradykinin-induced discharges were caused by excitation of the afferent C-fibres, because the discharges did not occur after pretreatment with capsaicin.

The results obtained from these studies seem to be in agreement with those of Matsumoto et al (1995): enalapril, but not imidapril, potentiated bradykinin-induced vascular leakage; there was no difference in the potentiation of substance P-induced vascular leakage between enalapril and imidapril, and no difference was observed between the potentiation of ozone-induced hypersensitivity in the airway by the two drugs. In contrast with our expectation, capsaicin-induced discharges were significantly depressed by enalaprilat but not by imidaprilat. Interestingly, enalaprilat depression was reversed by pretreatment with bradykinin B_2 receptor antagonist, Hoe-140. This and related findings suggest that an increase in bradykinin level or in the sensitivity of bradykinin receptor sites in the airways, or both of these, was involved in the enalaprilat depression of capsaicin response, and further, that there is a remarkable difference in bradykinin-related responses between enalapril and imidapril.

When bradykinin is considered here as a causative substance for cough potentiation arising as a result of administration of enalapril, some discrepancy seems to arise between the effects of enalaprilat on capsaicin discharges and on capsaicin-induced coughs. Capsaicin discharges were, however, rapidly desensitized and consequently poor in reproducibility (Fox et al 1993). This finding was also true in our experiment. Capsaicin showed dose-dependency in the narrow range 0·02 to 2 nmol kg^{-1} and capsaicin action was rapidly desensitized. The desensitization might be more facilitated under enalapril treatment. This property of capsaicin might, therefore, explain in part why enalapril failed to potentiate the capsaicin discharges, even though a small dose of enalaprilat tends to increase the discharges.

The activity of ACE in the degradation of tachykinins is 1000 times less potent than that of NEP (Turner et al 1985). In addition, captopril has very low activity in the inhibition of NEP (Turner et al 1985). In this context it is likely that ACE inhibitors might elevate the level of bradykinin more than tachykinin in living tissue.

There were no significant differences between the concentrations of the active metabolites of enalapril and imidapril in the lung, trachea and plasma of guinea-pigs (Kawashima et al 1994). In guinea-pigs, the ACE-inhibiting activities of imidaprilat were comparable with those of enalaprilat and ten times higher than those of captopril (Dr Narita, Tanabe Seiyaku, personal communication). The concentration of enalaprilat in the kidney after single and chronic dosing of enalapril was, however, much higher than that of imidaprilat (Kawashima et al 1994). The ACE activities in the kidney are very high. With regard to this, our preliminary study showed that enalapril increased the level of bradykinin in plasma, whereas imidapril tended to reduce it. Differences between the distribution to the kidney of enalapril and imidapril might, therefore, be one possible explanation of the results obtained from the electrophysiological studies.

The literature offers alternative or additional explanations of the electrophysiological data presented here. Wei et al (1990) reported that ACE has two active domains with different specificities and enzymatic properties (Wei et al 1992; Jaspard et al 1993). Although competitive ACE inhibitors generally have a lower K_d for the COOH-terminal domain than for the NH_2-terminal domain (Wei et al 1992), little is known about the relative potencies of imidapril for both domains.

Study of agonist selectivity, however, calculated as the ratio of inhibitory activity on angiotensin I-induced contraction to the potentiating activity on bradykinin-induced relaxation, showed that the potency of imidaprilat to potentiate bradykinin action is lower than that of enalaprilat (Okamura et al 1993), suggesting that enalaprilat causes greater increases in plasma bradykinin levels than does imidaprilat.

Little is known about how the potentiation of bradykinin responses and an increase in the bradykinin level trigger coughing. Bradykinin is known to stimulate the bronchial C-fibre to produce coughs (Kaufman et al 1980; Fuller et al 1987) and bradykinin-induced coughing and ACE inhibitor-induced coughing seem to be similar in that both types of cough are resistant to antitussives (Takahama et al 1990). In addition, bradykinin, when infused into the closed artery, potentiated capsaicin-induced coughs in guinea-pigs treated with a very low dose of enalapril. It is, therefore, likely that an increase in plasma bradykinin as a result of the ACE inhibitor might cause cough potentiation in guinea-pigs.

In an antagonistic study, the enalapril potentiation of coughs was depressed by an NK_1 blocker and by a bradykinin B_2 blocker. This might be because bradykinin stimulates the C-fibre terminal to release substance P in the airway (Kaufman et al 1980). In addition, a cyclooxygenase inhibitor has been reported to inhibit coughs associated with ACE inhibitors (McEwan et al 1990), suggesting involvement of prostaglandins in ACE inhibitor-induced coughs. This was also true in our preliminary study using guinea-pigs. The finding might be explained by the findings that: ACE inhibitor also stimulates tissue-production of prostaglandin (Galler et al 1982); bradykinin-induced tachykinin releases from the C-fibres are depressed by cyclooxygenase inhibitor (Geppetti et al 1991); and prostaglandins not only increase the sensitivity of the cough reflex (Choudry et al 1989) but also cause coughs (Roberts et al 1985). Mechanisms of enalapril-induced cough potentiation which might, therefore, be considered are: enalapril becomes more concentrated in the kidney, consequently increasing the level of bradykinin in plasma; bradykinin itself stimulates coughs; bradykinin releases substance P from the C-fibre terminals and also stimulates prostaglandins production to stimulate coughs.

In conclusion: imidapril was less potent in potentiating cough responses induced by capsaicin and citric acid in guinea-pigs than enalapril or captopril; bradykinin might be a key substance in the mechanisms of cough potentiation by ACE inhibitors in guinea-pigs.

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References

Cascieri, M. A., Bull, H. G., Mumfort, R. A., Patchett, A., Thornberry, N. A., Liang, T. (1984) Carboxylterminal tripeptidyl hydrolysis of substance P by purified rabbit lung angiotensin converting enzyme and the potentiation of substance P activity in vivo by captopril and MK-422. Mol. Pharmacol. 25: 287–293

Choudry, N. B., Fuller, R. W., Pride, N. B. (1989) Sensitivity of the human cough reflex: effect of inflammatory mediators, prostaglandin E_2, bradykinin and histamine. Am. Rev. Respir. Dis. 140: 137–141

Choudry, N. B., Fuller, R. W., Anderson, N., Karlsson, J.-A. (1990) Separation of cough and reflex bronchoconstriction by inhaled local anaesthetics. Eur. Respir. J. 3: 579–583

Coulter, D. M., Edwards, J. R. (1987) Cough associated with captopril and enalapril. Br. Med. J. 294: 1521–1523

Cushman, D. W., Cheung, H. S. (1971) Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. Biochem. Pharmacol. 20: 1637–1648

Forsberg, K., Karlsson, J.-A. (1986) Cough induced by stimulation of capsaicin-sensitive sensory neurons in conscious guinea-pigs, Acta Physiol. Scand. 128: 319–320

Fox, A. J., Barnes, P. J., Urban, L., Dray, A. (1993) An in vitro study of the properties of single vagal afferents innervating guinea-pig airways. J. Physiol. 469: 21–35

Fuchikami, J. (1992) Pharmacological Studies on Principles for Development of New Antitussives: Peripheral Mechanism of Cough Response and the Effect of Mai-Meu-Dong-Tang, a Chinese Herbal Medicine. Ph.D. Thesis, Kumamoto University, Japan

Fuchikami, J., Takahama, K., Kai, H., Miyata, T. (1990) Comparative study of the antitussive activity of Mai-Meu-Dong-Tang and codeine in normal and bronchitic guinea-pigs. Pharmacodyn. Ther. (Life Sci. Adv.) 9: 37–43

Fuller, R. W., Dixon, C. M. S., Cuss, F. M. C., Barnes, P. J. (1987) Bradykinin-induced bronchoconstriction in humans: mode of action. Am. Rev. Respir. Dis. 135: 176–180

Galler, M., Backenroth, R., Folkert, V. W., Schlondorrf, D. (1982) Effect of converting enzyme inhibitors on prostaglandin synthesis by isolated glomeruli and aortic strips from rats. J. Pharmacol. Exp. Ther. 220: 23–28

Geppetti, P., Bianco, E. D., Tramontana, M., Vigano, T., Folco, G. C., Maggi, C. A., Manzini, S., Fanciullacce, M. (1991) Arachidonic acid and bradykinin share a common pathway to release neuropeptide from capsaicin-sensitive sensory nerve fibers of the guinea-pig heart. J. Pharmacol. Exp. Ther. 259: 759–765

Hood, S., Nicholls, M. G., Gilchrist, N. (1987) Cough with angiotensin converting-enzyme inhibitors. New Zealand Med. J. 100: 6–7

Ichinose, M., Barnes, P. J. (1990) The effect of peptidase inhibitors on bradykinin-induced bronchoconstriction in guinea-pigs in vivo. Br. J. Pharmacol. 101: 77–80

Jaspard, E., Wei. L., Alhenc-Gelas, F. (1993) Differences in the properties and enzymatic specificities of the two active sites of angiotensin I-converting enzyme (Kininase II). J. Biol. Chem. 268: 9495–9503

Kase, Y., Seo, H., Oyama, Y., Sakata, M., Tomoda, K., Takahama, K., Hitoshi, T., Okano, Y., Miyata, T. (1982) A new method for evaluating mucolytic expectorant activity and its application. I. Methodology. Arzn. Forsch. 32: 368–373

Kaufman, M. P., Coleridge, H. M., Coleridge, J. C. G., Baker, D. G. (1980) Bradykinin stimulates afferent vagal C-fibres in intrapulmonary airways of dogs. J. Appl. Physiol. 48: 511–517

Kawashima, K., Mizuuchi, H., Yamanaka, K., Maki, T., Banno, K., Sato, T., Miyata, T., Takahama, K. (1994) Pharmacokinetics of imidapril, a new angiotensin-converting enzyme inhibitor, in guinea-pigs. Pharmacometrics 48: 371–381

Matsumoto, Y., Murata, T., Kikura, K., Koshida, T., Kaminuma, O., Naito, K., Kikura, K., Tsuzurahara, K. (1995) The effect of ACE inhibitors on bradykinin-induced microvascular leakage in guinea-pig airways. Japan. J. Pharmacol. 67 (Suppl. I): 313p

McEwan, J. R., Choudry, N. B., Fuller, R. W. (1990) The effect of sulinduc on the abnormal cough reflex associated with dry cough. J. Pharmacol. Exp. Ther. 255: 161–164

Nakashima, M., Hashimoto, H., Hayase, K., Hamajima, K. (1984) Phase I trial of a single oral dose of MK-421, a new angiotensin I converting enzyme inhibitor, in healthy subjects. First report. Yakuri To Chiryo 12: 3357–3374

Okamura, T., Kitamura, Y., Kimura, T., Toda, N. (1993) Comparison of selective actions of imidaprilat and enalaprilat on the response to angiotensin I and bradykinin in isolated dog blood vessels. Pharmacometrics 46: 427–436

Oomura, I., Maki, E., Naruse, T., Chen, C.-S., Ideda, N., Asami, T. (1985) Effect of single and repeated oral administration of MK-421 and captopril on blood pressure in normotensive and experimental hypertensive rats. Folia Pharmacol. Japon. 86: 293–302

Roberts, A. M., Shultz, H. D., Green, J. F., Armstrong, D. J., Kaufman, M. P., Coleridge, H. M., Coleridge, J. C. G. (1985) Reflex tracheal constriction evoked in dogs by bronchodilator prostaglandin E_2 and I_2. J. Appl. Physiol. 58: 1823–1831

Sakata, K., Takahama, K., Kai, H., Isohama, Y., Miyata, T. (1993) Studies on mechanisms of cough augmentation in SO_2-exposed guinea-pigs. Japan. J. Pharmacol. 61 (Suppl. I): 80p

Saria, A., Martling, C.-R., Yan, Z. (1988) Release of multiple tachykinins from capsaicin-sensitive sensory nerves in the lung by bradykinin, histamine, dimethylphenylpiperazinium, and vagal nerve stimulation. Am. Rev. Respir. Dis. 137: 1330–1335

Sasaguri, M., Ideishi, M., Hiroki, T., Arakawa, K. (1994) Comparative preliminary test of imidapril and other ACE inhibitors on cough production. Shinyaku To Rinsho 43: 2467–2471

Skidgel, R. A., Erdos, E. G. (1987) The broad substrate specificity of human angiotensin I converting enzyme. Clin. Exp. Hypertens. A9: 243–259

Takahama, K., Fuchikami, J., Kai, H., Miyata, T. (1990) The effect of Mai-Meu-Dong-Tang, a Chinese herbal medicine, on the cough augmented by angiotensin-converting enzyme inhibitors. J. Med. Pharm. Soc. (Wakan-Yaku) 7: 310–311

Takahama, K., Fuchikami, J., Suzuki, A., Tabata, T., Kai, H., Miyata, T. (1993) Differences in the mode of cough augmentation by four angiotensin-converting enzyme inhibitors in guinea-pigs. J. Pharm. Pharmacol. 45: 1003–1005

Turner, A. J., Matsas, R., Kenny, A. J. (1985) Commentary. Are there neuropeptidase-specific peptidases? Biochem. Pharmacol. 34: 1347–1356

Wei, L., Alhenc-Gelas, F., Corvol, P., Clauser, E. (1990) The two homologous domains of human angiotensin I converting enzyme are both catalytic active. J. Biol. Chem. 266: 9002–9008

Wei, L., Clauser, E., Alhenc-Gelas, F., Corvol, P. (1992) The two homologous domains of human angiotensin I-converting enzyme interact differently with competitive inhibitors. J. Biol. Chem. 267: 13398–13405

Yang, H. Y. T., Erdos, E. G., Levin, Y. (1970) A dipeptidyl carboxypeptidase that converts angiotensin I and inactivates bradykinin. Biochem. Biophys. Acta. 214: 374–376

Yokosawa, H., Endo, S., Ohgaki, Y., Maeyama, J., Ishii, S. (1985) Hydrolysis of substance P and its analogs by angiotensin-converting enzyme from rat lung. Characterization of endopeptidase activity of the enzyme. J. Biochem. 98: 1293–1299