Responses of airway rapidly adapting receptors to bradykinin before and after administration of enalapril in rabbits

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1. The present study was performed in anaesthetised, artificially ventilated, open-chested rabbits to examine whether (a) the rapidly adapting receptors of the airways were stimulated by exogenously administered bradykinin, and (b) if this sensitivity could be enhanced by the angiotensin-converting-enzyme inhibitor, enalapril.

2. Rapidly adapting receptor activity \( (n=8) \) was recorded from the cervical vagus. Bradykinin was injected intravenously \( (0.25-1.0 \, \mu g/kg) \) and a dose-response curve relating receptor activity to bradykinin was elicited. In the control state, the threshold dose of bradykinin required for stimulation of rapidly adapting receptors was \( 0.53 \pm 0.11 \, \mu g/kg \). Five minutes after the administration of enalapril maleate \( (2 \, mg \text{ intravenously}) \), the dose-response curve was shifted to the left significantly \( (P<0.01) \).

3. In seven other rapidly adapting receptors, enalapril \( (2 \, mg) \) increased the resting activity significantly \( (P<0.05) \) over a period of 60 min. This increase was significantly different from the spontaneous variation in neural activity of rapidly adapting receptors \( (n=7) \) recorded over a period of 60 min.

4. Bradykinin either alone \( (0.25-1.0 \, \mu g/kg) \) or in the presence of enalapril did not stimulate the slowly adapting receptors \( (n=5) \) of the airways.

5. These results show that (a) exogenous bradykinin stimulates the rapidly adapting receptors, (b) the sensitivity of rapidly adapting receptors to bradykinin is enhanced by enalapril and (c) enalapril increases the resting activity of rapidly adapting receptors. It is suggested that the cough reported after the administration of enalapril may be due to stimulation of rapidly adapting receptors of the airways.

INTRODUCTION

Angiotensin-converting enzyme (ACE) inhibitors are often prescribed for the treatment of hypertension and heart failure. Although ACE inhibitors are tolerated relatively well by patients, a side effect which has been reported with increasing frequency is a troublesome cough \([1-3]\). It has been reported in 2–8% of patients taking enalapril and in 0.2–1.1% taking captopril \([2]\).

Several chemical mechanisms have been postulated to explain this phenomenon. For instance, it has been suggested that the cough is due to stimulation of sensory receptors in the lungs which are activated by chemicals that accumulate after ACE inhibition. One agent which has been implicated in the cough response is bradykinin \([4]\). After ACE inhibition, bradykinin is believed to accumulate in the lung due to the inhibition of kininase 1, which is one agent which has been implicated in the cough response is bradykinin \([4]\). After ACE inhibition, bradykinin is believed to accumulate in the lung due to the inhibition of kininase II which is responsible for its breakdown \([5]\). However, there is no experimental study which has directly compared the responses of sensory receptors of the airways to bradykinin before and after administration of ACE inhibitors.

Recent studies in dogs and rabbits have shown that pulmonary venous congestion produced by partial obstruction of the mitral valve markedly stimulated the rapidly adapting receptors of the airways \([6, 7]\). This response was potentiated when the oncotic pressure of plasma was reduced by plasmapheresis \([7, 8]\). Thus, it was proposed that the natural stimulus for the rapidly adapting receptor could be fluid fluxes across the bronchial vasculature \([9]\).

Bradykinin, which is a powerful vasodilator, increases the permeability of bronchial venules when administered into the circulation \([10, 11]\). It also increases the tone in the smooth muscle of the large airways \([12]\). Administration of ACE inhibitors is likely to facilitate a fluid flux across the bronchial vasculature by increasing the concentration of endogenous bradykinin. The present investigation was undertaken to examine the effect of the ACE inhibitor enalapril upon the activity of rapidly adapting receptors which are also known to mediate a cough reflex \([13]\). Two specific issues were examined: (i) the effect of enalapril upon the sensitivity of rapidly adapting receptors to exogenous bradykinin; (ii) the effect of enalapril upon the activity of rapidly adapting receptors to exogenous bradykinin before and after administration of ACE inhibitors.

Key words: bradykinin, enalapril, vagal sensory receptors.

Abbreviations: ACE, angiotensin-converting enzyme; \( P_{CO_2} \), partial pressure of \( CO_2; P_{O_2} \), partial pressure of \( O_2 \).

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adapting receptors. The responses of the pulmonary slowly adapting receptors to bradykinin were examined as a subsidiary investigation.

METHODS

The study was performed on 27 rabbits weighing between 3 and 5 kg. They were anaesthetized with an intravenous injection of sodium pentobarbitone (Somnolol; MTC Pharmaceuticals, Cambridge, Ontario, Canada) 30 mg/kg intravenously through a cannula inserted into the marginal ear vein. Surgical anaesthesia was maintained during the experiment by further doses of the anaesthetic (12 mg h\(^{-1}\) kg\(^{-1}\)). A tracheostomy was performed in the neck, and a cannula with a side arm was inserted. A polyethylene tube (internal diameter 0.86 mm; Intramedic Polyethylene Tubing, Clay Adams Inc., Parsippany, NJ, U.S.A.) was placed in the side arm of the tracheal cannula and was used for recording airway pressure. The animals were ventilated artificially with a Harvard ventilator (model 607; Harvard Instruments, Millis, MA, U.S.A.) at a rate of 22 breaths/min with a tidal volume of 12–15 ml/kg. The inspired gas was supplemented with O\(_2\) and the arterial partial pressure of O\(_2\) (P\(_{O2}\)) was maintained above 100 mmHg. A thoracotomy was performed in the midline. The expiratory outlet from the ventilator was immersed in 2 cm water.

The temperature of the animals, measured using a thermistor (model 43TD; Yellow Springs Instruments Co, Yellow Springs OH, U.S.A.) inserted into the rectum, was maintained at 37±1°C by means of heating lamps.

Cannulae (inner diameter 0.86 mm; Intramedic Polyethylene Tubing) were inserted into the right femoral artery, the right femoral vein and the left atrium. The cannula in the femoral vein was used for administering infusions and drugs. The cannula in the femoral artery was used for monitoring intracarotid pressure and that in the left atrium for recording left atrial pressure. Samples of blood were collected from the arterial cannula periodically for blood gas measurements. Arterial partial pressure of CO\(_2\) (P\(_{CO2}\)) and pH were maintained in the normal range by adjusting the tidal volume and by infusing 7.5% (w/v) sodium bicarbonate.

The cannulae for recording pressures were connected to transducers (model P23 DB; Statham Instruments Ltd, Hato Rey, Puerto Rico), the outputs of which were amplified and recorded on light-sensitive paper (model VR 12; Electronics for Medicine/Honeywell, Pleasantville, NY, U.S.A.). The zero values for arterial pressures were obtained post mortem after exposure of the tip of the cannula to the atmosphere. Mean pressure was obtained electronically from the pulsatile pressures.

Recording of action potentials from the vagus nerve

The left cervical vagus nerve was separated from the carotid sheath, and action potentials originating from slowly adapting stretch receptors and rapidly adapting receptors were recorded using bipolar Ag/AgCl electrodes. Single-fibre activity was recorded from slips of the vagus. The outputs from the electrodes were fed into a preamplifier and recorded on light-sensitive paper as described above. The neural activity in the fibres was counted electronically using a pulse discriminator described previously [14]. The output from the recorder was used for this purpose. The right vagus was left intact.

The slowly adapting receptors were identified by their characteristic respiratory rhythm and their slow rates of adaptation to a sustained inflation of the lungs. The rapidly adapting receptors were identified by their rapid adaptation to a sustained inflation of the lungs. For this purpose, the expiratory outlet of the respiratory pump was occluded at end-expiration and the lungs were inflated for three ventilatory cycles. At the peak of the third cycle, the pump was switched off and the inflation was maintained by occluding the endotracheal tube approximately 5 cm away from the animal. An adaptation index was calculated as described previously [6] and only those fibres with an adaptation index greater than 70% were accepted as originating from rapidly adapting receptors [15, 16]. At the conclusion of each experiment, the location of each receptor was established by punctate stimulation on the external surface of the airways using a blunt glass rod with a tip diameter of 3 mm.

Measurement of conduction velocity of afferent fibres of the vagus

The conduction velocity was measured using conventional techniques [17]. For this purpose, a pair of stimulating electrodes was placed underneath the vagus near the thoracic inlet. Stimuli (strength 2–6 V, duration 15 ms) were delivered from a stimulator (model SD9B; Grass Instruments, Quincy, MA, U.S.A.). The conduction velocity was calculated from the latency of the evoked response and the distance between the stimulating and recording electrodes.

Drugs

A stock solution of bradykinin acetate (10 mg/ml) (molecular mass 1060.25 Da; Sigma, St Louis, MO, U.S.A.) in 150 mmol/l NaCl was prepared and stored at −5°C. It was diluted on the day of the experiment to give a concentration of 10 μg/ml. During the experimental period, the solution was protected from light.

The solution of enalapril (enalapril maleate, mole-
cular mass 492.53 Da, Merck Frosst, Montreal, Canada) was prepared fresh for each experiment (2 mg of the base was dissolved in 2 ml of 150 mmol/l NaCl).

Experimental protocols

After isolating and identifying pulmonary afferent fibres as described above, the preparation was left to stabilize for 10 min. Thereafter, it was subjected to one of the following protocols.

Protocol 1: effect of bolus injections of bradykinin on the activity of rapidly adapting receptors before and after the administration of enalapril. Only one receptor was examined in each rabbit. The neural activity was recorded for an initial control period of 1 min. Then, graded doses of bradykinin (0.25, 0.5 and 1.0 μg/kg) were injected intravenously. Each dose of bradykinin was flushed with 1.5 ml of 0.15 mol/l NaCl. The total volume injected at any instant was not more than 2 ml. The time of injection was recorded with a signal marker given at the instance of the flush. The effect of bradykinin on the afferent activity was examined by recording neural activity for a period of 1 min. Between each injection, an interval of 10 min was allowed for the neural activity and the cardiovascular parameters to return to control values. Before each dose, an equal volume of 0.15 mol/l NaCl was injected to determine the effect of the vehicle on the afferent activity.

In each of the rabbits examined above, once the afferent activity returned to the control values, a fixed dose of enalapril (2 mg) was injected intravenously. Five minutes later, protocol 1 was repeated.

The threshold dose of bradykinin required for the activation of rapidly adapting receptors was established in the following manner. The action potentials in the control period were counted in six consecutive 10 s periods and were expressed as the average activity per 10 s. A similar calculation was made during the 60 s after the injection of bradykinin. If the neural activity in any consecutive 10 s period after injection of bradykinin had increased to a value greater than two times the SD from the mean activity in the control period, that dose was considered as the threshold. The change in the threshold dose of bradykinin after the administration of enalapril was also estimated in the same manner as described above.

The individual responses were calculated either in terms of the ‘total activity’ or the ‘peak activity’. ‘Total activity’ represented the cumulative number of action potentials during the 1 min recording periods. ‘Peak activity’ was the highest activity during one ventilatory cycle within each recording period and was expressed as impulses/s.

Protocol 2: effect of bolus injections of bradykinin on the activity of slowly adapting receptors before and after the administration of enalapril. The effect of bradykinin on the activity of slowly adapting receptors was examined using the same protocol as described above.

Protocol 3: effect of enalapril on the activity of rapidly adapting receptors. This protocol examined the effect of enalapril alone on the activity of rapidly adapting receptors over a period of 60 min. The neural activity was recorded for an initial control period of 15 min. Then, enalapril (2 mg) was injected intravenously and the neural activity was recorded for 60 min.

In a separate group of rapidly adapting receptors (time controls), the natural variation in receptor activity was examined without any interventions over a period of 60 min.

Analysis of data

The dose-response curves relating receptor activity and different doses of bradykinin elicited before and after the administration of enalapril were compared using analysis of co-variance.

Dose–response curves were also elicited for the blood pressure and heart rate responses to bradykinin before and after enalapril and were compared using analysis of co-variance. For the changes in blood pressure and heart rate, values representing the greatest deviation from the control after a bolus injection of bradykinin were considered for analysis.

The influence of enalapril on rapidly adapting receptor activity was analysed in two ways: (a) the activities recorded over a period of 60 min after the injection of enalapril were averaged and compared with the average activity recorded during the control period (15 min) using a paired t-test, and (b) the activities recorded after administration of enalapril were compared with those in a similar number of receptors which were studied as time controls.

For this purpose, the activity in the time controls over a 15 min period was taken as the baseline and the activities in three successive 15 min periods were taken as repeated measures of the same parameter. The data from the receptors in animals treated with enalapril were analysed in the same manner, i.e. the first 15 min were taken as the baseline and next three successive 15 min periods after enalapril administration were taken as repeated measures. Data were analysed by a two-way analysis of variance with repeated measures, with time and drug administration as factors contributing to the variance.

Group data were expressed as means ± SEM. A P value < 0.05 was accepted as indicative of statistical significance.

RESULTS

In the 27 rabbits studied, the resting heart rate, mean arterial blood pressure, peak airway pressure and mean left atrial pressure were 193 ± 6.6 beats/min, 81.0 ± 4.5 mmHg, 0.97 ± 0.07 mmHg and 4.8 ± 0.5
mmHg, respectively. The arterial blood pH, $P_{CO_2}$ and $PO_2$ were $7.37 \pm 0.1$, $38.7 \pm 1.5$ mmHg ($5.2 \pm 0.2$ kPa) and $185 \pm 16$ mmHg ($24.7 \pm 2.1$ kPa), respectively. Injection of the vehicle alone did not stimulate any of the vagal afferents.

Protocol I: effect of bolus injections of bradykinin on the activity of rapidly adapting receptors before and after the administration of enalapril

Eight rapidly adapting receptors were investigated in eight rabbits. All receptors exhibited spontaneous activity. Their conduction velocity was $14.2 \pm 2.0$ m/s. The average threshold dose of bradykinin required for the activation of the rapidly adapting receptors was found to be $0.53 \pm 0.11$ µg/kg. The threshold was $0.25$ µg/kg in three units, $0.5$ µg/kg in three units and $1.0$ µg/kg in the remaining two units. With increasing doses of bradykinin, there was a graded response in the total and the peak activities. An example is shown in Fig. 1. These results are summarized in Table 1. The dose–response curves elicited are shown in Fig. 2.

The changes observed in the peak airway pressures measured during the injection of graded doses of bradykinin are shown in Table 2. Whereas low doses of bradykinin ($0.25$ and $0.5$ µg/kg) did not produce any significant change in the peak airway pressure, a slight increase (approximately $0.08$ kPa) was observed with a dose of $1.0$ µg/kg. The control mean arterial blood pressures before and after administration of enalapril were $95 \pm 5$ and $88 \pm 6$ mmHg, respectively ($P>0.05$). There was a progressive decrease in mean arterial blood pressure as well as in heart rate with increasing doses of bradykinin. These results are presented in Table 2. Data on the effect of bradykinin on blood pressure in rabbits after bilateral vagal section have been deposited as Clinical Science Table 92/3 with the Librarian, Royal Society of Medicine, 1 Wimpole Street, London W1M 8AE, U.K., from whom copies are available on request.

Responses after the administration of enalapril.

After the administration of enalapril, an increase in the resting activity (total activity) was seen in seven of the eight receptors examined. Overall, this increase was statistically significant ($P<0.05$, paired t-test). In addition, the threshold dose of bradykinin required for activation of the rapidly adapting receptors was significantly reduced ($P<0.05$). The threshold dose was $0.25$ µg/kg in all eight units. An example of a rapidly adapting receptor showing an increase in sensitivity is shown in Fig. 1. Overall, the sensitivity of the receptors to bradykinin was enhanced. The dose–response curves relating peak
and total activities to increasing doses of bradykinin are shown in Figs. 2(a) and 2(b), respectively. Each curve was shifted significantly to the left after administration of enalapril. These results are summarized in Table 2.

The changes observed in the peak airway pressures measured during the injections of graded doses of bradykinin are shown in Table 2. When these values were compared with those recorded before the administration of enalapril, there was no significant difference (analysis of co-variance, \(P<0.05\)). After enalapril, there was a potentiation of the cardiovascular responses to bradykinin. For a given dose, the falls in heart rate and mean arterial blood pressure were greater compared with those recorded before the administration of enalapril. These results are presented in Table 2.

Location. Of the eight rapidly adapting receptors investigated, four were located in the lobar bronchus, one in the principal bronchus and three in the lung parenchyma less than 1 cm from the hilum of the lung.

Protocol 2: effect of bolus injections of bradykinin on the activity of slowly adapting receptors before and after the administration of enalapril

Five slowly adapting receptors were investigated in five rabbits. Their conduction velocity was 22.2 ± 3.0 m/s. Of the five receptors, three were located in the lobar bronchus and two in the lung parenchyma less than 1 cm from the hilum of the lung.

Bradykinin failed to stimulate the slowly adapting receptors significantly in the control state. There were no significant changes in the peak and total activities after injections of bradykinin. This behaviour was not significantly altered after the administration of enalapril. These results are summarized in Table 1.

Protocol 3: effect of enalapril on the activity of rapidly adapting receptors

The effects of enalapril on the activity of rapidly adapting receptors was examined in seven units in seven rabbits. In six of these units, there was an increase in activity after the administration of enalapril. In the remaining unit, the activity was not increased. There was a variation in the pattern of responses to the administration of enalapril. Three examples are shown in Fig. 3. Overall, there was a significant stimulation of the receptors after administration of enalapril from a control value of 192 ± 25 to 231 ± 26 impulses/min \(P<0.05\).

The spontaneous variation in the activity of rapidly adapting receptors over 60 min was examined in seven units (time controls). In order to establish whether administration of enalapril after 15 min influenced the activity over the following 45 min, the data from both sets of receptors were compared using analysis of variance (see the Methods section). It was found that the variation in activity over time was not significant in time controls and that there was a significant increase in activity in receptors treated with enalapril \((F=3.88, P<0.05, \text{Fig. 4})\).

### Table 1. Responses of the rapidly adapting receptors (RAR) and slowly adapting receptors (SAR) to incremental intravenous doses of bradykinin before and after intravenous administration of enalapril (2mg). Results are expressed as peak activity and total activity (all means ±SEM). See the Methods section for an explanation. In each row, the symbols indicate the following: * significantly different from control and 0.25 μg of bradykinin/kg; ** significantly different from control \(P<0.05\) for both, analysis of variance; † significantly different from corresponding values before enalapril (paired t-test).

<table>
<thead>
<tr>
<th>Location</th>
<th>Control</th>
<th>Bradykinin (μg/kg)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0.25</td>
<td>0.5</td>
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<tr>
<td>RAR (n=8)</td>
<td></td>
<td></td>
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<tr>
<td>Before enalapril</td>
<td></td>
<td></td>
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<tr>
<td>Total activity (impulses/min)</td>
<td>273 ± 50</td>
<td>281 ± 37</td>
</tr>
<tr>
<td>Peak activity (impulses/s)</td>
<td>58 ± 15</td>
<td>81 ± 14</td>
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<tr>
<td>After enalapril</td>
<td></td>
<td></td>
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<tr>
<td>Total activity (impulses/min)</td>
<td>345 ± 46†</td>
<td>549 ± 88</td>
</tr>
<tr>
<td>Peak activity (impulses/s)</td>
<td>74 ± 12†</td>
<td>146 ± 22</td>
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<tr>
<td>SAR (n=5)</td>
<td></td>
<td></td>
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<tr>
<td>Before enalapril</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total activity (impulses/min)</td>
<td>1426 ± 242</td>
<td>1436 ± 268</td>
</tr>
<tr>
<td>Peak activity (impulses/s)</td>
<td>247 ± 42</td>
<td>249 ± 47</td>
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<tr>
<td>After enalapril</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total activity (impulses/min)</td>
<td>1466 ± 258</td>
<td>1472 ± 279</td>
</tr>
<tr>
<td>Peak activity (impulses/s)</td>
<td>254 ± 46</td>
<td>255 ± 48</td>
</tr>
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</table>
DISCUSSION

ACE inhibitors retard the conversion of angiotensin I to angiotensin II, and are used widely in the management of patients with heart failure and hypertension. Since ACE is identical with kininase II [5], ACE inhibitors will also attenuate the breakdown of bradykinin [18]. Thus it is likely that ACE inhibitors would potentiate the physiological effects of bradykinin. Indeed, it has been shown that enalapril enhances the wheal and flare formation which follows the intradermal administration of bradykinin [19].

The lungs clear a number of hormones and prohormones and thereby regulate their concentrations in arterial blood [20, 21]. The presence of kininase II in the lung ensures that bradykinin released locally is metabolized rapidly in the lung [22]. In addition, circulating bradykinin is degraded almost completely during a single passage through the pulmonary vascular bed [22]. Since the production and release of bradykinin in the lungs is enhanced during attacks of asthma and during anaphylaxis [23], it has been suggested that 'endogenous' bradykinin released in this way may stimulate vagal afferents from the lungs and participate in the symptomatology associated with these conditions [24].

Previous investigations in dogs [6, 8, 25] and rabbits [7] have shown that the rapidly adapting receptors of the airways are exquisitely sensitive to fluid fluxes across the bronchial vasculature. Since bradykinin increases bronchovenular permeability [10, 11], it was hypothesized that these receptors would be particularly sensitive to bradykinin released 'locally' in the lung.

Responses of rapidly adapting receptors to bradykinin

In the present study, bradykinin stimulated all the rapidly adapting receptors examined. This finding differs partially from an earlier observation in dogs [26], where it was reported that bradykinin stimulated 13 of the 23 rapidly adapting receptors when it was injected into the left atrium or bronchial artery. Also, when injected into the right atrium, bradykinin stimulated only three of nine rapidly adapting receptors [26]. Since the respective doses of bradykinin used in the two studies were similar, it appears that there may be a species variation in the sensitivity of rapidly adapting receptors to bradykinin. This variation may be due, in part, to a difference in the diffusion path in the two species.

The mechanism of action of bradykinin on rapidly adapting receptors remains to be resolved. It may have a direct effect on the receptor itself or an indirect one through changes in bronchial vascular permeability and/or changes in tone of the large airways. In the present study, it was shown that the activity of the rapidly adapting receptors was enhanced before bradykinin produced any significant change in peak airway pressure. Although this observation suggests that the responses of the receptors to bradykinin (at low doses) may be due to an increased vascular permeability or a direct effect of bradykinin on the receptor itself, a definitive conclusion must await further studies.

Effect of enalapril on rapidly adapting receptors

Enalapril sensitized the rapidly adapting receptors to injections of bradykinin as indicated by the changes in the slopes and intercepts of the stimulus–response curves to bradykinin. In addition, enalapril increased the resting activity of rapidly adapting receptors, the effect being evident within 15 min of intravenous administration of the drug. Although the pattern of this activation varied in the individual units examined, the overall increase was significant and could not be explained by any natural variation.
in the activity of these receptors. The response observed is unlikely to be a non-specific effect, since enalapril failed to stimulate the slowly adapting receptors. Taken collectively, these findings support the hypothesis that enalapril exerts its effect upon the rapidly adapting receptors by potentiating the influence of endogenous bradykinin.

All these effects upon receptor activity appear to be limited to the rapidly adapting receptors. The slowly adapting receptors are not activated by either bradykinin or enalapril. Similar findings have been reported previously in the dog with respect to bradykinin [26].

Speculation on the clinical significance

Cough is an adverse of ACE inhibitors [2, 3, 29], which is believed to be secondary to their influence on the metabolism of prostaglandins [29], bradykinin and other tachykinins [30]. While there is general agreement on the effect of ACE inhibitors on kininase II, the effect of these compounds on prostaglandins appears to be structure-specific [31]. For instance, in both aortic endothelial cells and renal medullary cells, captopril, which contains a thiol group, increases prostaglandin synthesis. Enalapril, which has no thiol group, has no effect on prostaglandin synthesis [31]. The data from human studies do not provide definitive evidence of a role for prostaglandins in ACE-inhibitor-induced cough. For instance, McEwan et al. [32] have claimed that ACE-inhibitor-induced cough was attenuated by sulindac. This claim is based on a study of six patients, two of whom were on enalapril and four on captopril. In contrast, Gilchrist et al. [33] found no significant improvement in cough with sulindac (n=8). Both groups of investigators examined hypertensive patients. The former investigation was a randomized placebo-controlled double-blind study, whereas the latter was a randomized placebo-controlled double-blind cross-over study. Thus the potential role of prostaglandins in this side effect remains to be defined.

With respect to the role of bradykinin in the ACE-inhibitor-induced cough, two lines of evidence could be examined. The first is the effect of bradykinin on pulmonary receptors in experimental animals, and the second is the effect of ACE inhibitors on bradykinin-induced responses in humans. In animals, at doses employed in the present study, bradykinin activates both rapidly adapting receptors (see above) and bronchial C fibre receptors [12, 26]. There is a considerable body of evidence to suggest that the rapidly adapting receptors mediate a reflex cough. It has also been suggested that bronchial

| Table 2. Changes in heart rate, mean arterial blood pressure (MABP) and peak airway pressure after incremental intravenous doses of bradykinin before and after intravenous administration of enalapril (2 mg) in rabbits (n=8). In each row, the symbols indicate the following: *, significantly different from control and 0.25 μg of bradykinin/kg; **, significantly different from control; ***significantly different from all the other values in the row (P<0.05 for all three, analysis of variance). †, Significantly different from corresponding values before enalapril (P<0.05, paired t-test). There was a significant change in elevation of the regression lines relating both heart rate and blood pressure to the doses of bradykinin (see the Methods section). |
|-----------------|-----------------|-----------------|-----------------|
|                 | Control         | Bradykinin (μg/kg) | 0.25 | 0.5 | 1.0 |
| Before enalapril |                 |                 |      |     |     |
| Heart rate (beats/min) | 219 ± 5 | 213 ± 6 | 194 ± 15 | 184 ± 16* |
| MABP (mmHg)      | 95 ± 5 | 73 ± 6 | 67 ± 7** | 61 ± 6*** |
| Peak airway pressure (kPa) | 0.92 ± 0.07 | 0.95 ± 0.08 | 0.95 ± 0.07 | 0.99 ± 0.1** |
| After enalapril  |                 |                 |      |     |     |
| Heart rate (beats/min) | 218 ± 5 | 162 ± 20 | 161 ± 19 | 144 ± 20 |
| MABP (mmHg)      | 88 ± 6*** | 46 ± 7 | 52 ± 6 | 45 ± 3 |
| Peak airway pressure (kPa) | 0.99 ± 0.07† | 1.01 ± 0.07 | 1.05 ± 0.07 | 1.07 ± 0.1** |

Dose and the time course for the action of enalapril

Enalapril, being a ‘prodrug’, is de-esterified to its active form enalaprilat in the liver. Even though de-esterification is required for complete expression of ACE inhibitor activity, enalapril has been shown to have an inherent effect (without de-esterification) in augmenting the responses to bradykinin in the isolated guinea pig ileal preparation [27]. Thus, the responses of rapidly adapting receptor activity obtained in the present study may be in part due to the inherent effect of enalapril and in part due to its conversion to enalaprilat. In a recent study in rats [28], it was reported that kininase II was inhibited in blood samples collected 5 min after the intravenous administration of enalaprilat (0.2 mg/kg). In the present study in rabbits, enalapril (2 mg) was administered as a single intravenous bolus injection and the rapidly adapting receptors were found to be activated within 15 min. Although the proportion of enalapril converted to enalaprilat in the rabbit within this period of an intravenous injection is not known, it appears that even a modest degree of conversion is sufficient to produce a significant biological effect.
C fibre receptors could cause a similar effect [13]. Thus, based upon studies in experimental animals, it could be concluded that bradykinin is a tussigenic stimulus. The data from human studies are not definitive. For instance, Dixon et al. [34] showed that ramipril caused no change in lung function or airway reactivity to bradykinin in mild asthmatics. The incidence of cough was not reported. However, Katsumata et al. [35] in a controlled study showed that the incidence of cough in response to bradykinin was increased after the administration of both captopril and enalapril. On the basis of this evidence, a role for bradykinin cannot be excluded.

Finally, there is no direct evidence that the effects of tachykinins, such as substance P, and other peptides, such as calcitonin-gene-related peptide, on pulmonary receptors are influenced by ACE inhibitors. However, there is some circumstantial evidence in patients with mild asthma [29] and in normal subjects [29, 30] that the tussigenic effect of capsaicin (which leads to the release of substance P) is enhanced after the administration of ACE inhibitors. Further studies on receptor activities would be of interest. In summary, the findings presented in this paper support the claim that rapidly adapting receptors are stimulated by bradykinin and that this effect is enhanced by enalapril. It is suggested that this phenomenon may be one of the mechanisms involved in the ACE-inhibitor-induced cough response observed in man.

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