Angiotensin converting enzyme inhibitors potentiate the bronchoconstriction induced by substance P in the guinea-pig

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- 1 The effects of intravenous captopril and enalaprilic acid on the increase in pulmonary inflation pressure induced by different bronchoconstrictor agents were evaluated in the anaesthetized guinea-pig.
- 2 Captopril and enalaprilic acid ($1.6\text{--}200\ \mu\text{g kg}^{-1}$) enhanced dose-dependently the bronchoconstriction (BC) induced by substance P. The threshold effective dose was $1.6\ \mu\text{g kg}^{-1}$ and maximal potentiation over the control response was more than 400% for both agents. Enalaprilic acid was also assayed for serum and lung angiotensin converting enzyme (ACE) inhibition in anaesthetized guinea-pigs. This drug produced a dose-dependent inhibition of ACE in both tissues, with ED_{50} s of 7.6 and $9.4\ \mu\text{g kg}^{-1}$, respectively: this inhibitory activity was positively correlated to substance P potentiation.
- 3 Captopril ($8\text{--}1000\ \mu\text{g kg}^{-1}$) enhanced dose-dependently the BC induced by capsaicin. The threshold effective dose was $40\ \mu\text{g kg}^{-1}$ and maximal potentiation about 90%.
- 4 Captopril ($200\text{--}1000\ \mu\text{g kg}^{-1}$) did not affect BC induced by bradykinin. However, this response was markedly enhanced (about 200%) by captopril $200\ \mu\text{g kg}^{-1}$ in propranolol-pretreated animals.
- 5 Captopril and enalaprilic acid ($200\text{--}1000\ \mu\text{g kg}^{-1}$) slightly (20–40%) but significantly enhanced the BC induced by 5-hydroxytryptamine. However, this response was potentiated to the same extent by a dose of prazosin, which produced a degree of hypotension similar to that observed after administration of the ACE inhibitors.
- 6 In conclusion, ACE inhibitors potentiate the BC induced by substance P and, to a minor extent, that induced by capsaicin in the anaesthetized guinea-pig. Potentiation of substance P is well correlated with ACE inhibition in guinea-pig serum and lungs. These experimental results may offer a mechanistic interpretation of cough and bronchial hyperreactivity observed in patients receiving treatment with ACE inhibitors.

Introduction

Angiotensin converting enzyme (ACE) inhibitors are being used increasingly in the treatment of hypertension and heart failure. One of the most recently recognized side-effects of these agents is the occurrence of a persistent dry cough (Coulter & Edwards, 1987), which is apparently associated with bronchial hyperreactivity (Bucknall *et al.*, 1988). Both cough and hyperreactivity are prominent features of bronchial asthma. Therefore these symptoms may result from the 'unmasking of asthma' by ACE inhibitors, as previously suggested by Salena (1986). ACE is identical with kininase II, an enzyme that degrades bradykinin, and this kinin may activate local axon reflexes by stimulating C-fibre afferent nerve endings, ultimately leading to bronchoconstriction (BC) and airway inflammation (Barnes, 1986). Moreover, ACE is capable of catalyzing the metabolism of substance P, one of the inflammatory and bronchoconstrictor tachykinins widely distributed throughout the respiratory system of various species (Cascieri *et al.*, 1984). In view of the above, we examined the effects of captopril and enalaprilic acid (MK 422, the active parent diacid of enalapril) on the response induced by different bronchoconstrictor agents: substance P, capsaicin (the pungent principle of hot peppers of the genus *Capsicum*, which selectively releases neuropeptides, including substance P, from C-fibre afferents), bradykinin and 5-hydroxytryptamine (5-HT).

Methods

Male Dunkin-Hartley guinea-pigs, body weight 300–400 g, from Rodentia, Torre Pallavicina (Bergamo), were used. The animals were anaesthetized with ethyl urethane ($1.2\ \text{g kg}^{-1}$

i.p.). A tracheal cannula was connected to a Rodent Ventilator (U. Basile, Italy) calibrated at 3–4 ml and 60 r.p.m. and spontaneous respiration was abolished by gallamine $3\ \text{mg kg}^{-1}$ i.v. Pulmonary inflation pressure (PIP) and mean blood pressure were measured via Bentley Trantec 800 transducers, connected to a side-arm of the tracheal cannula and to a carotid artery respectively, and recorded on a Gemini recorder (U. Basile). After an equilibration period of at least 20 min, bronchoconstrictor agents were injected intravenously at doses aimed at producing an increase in PIP of approximately $10\ \text{cmH}_2\text{O}$. Capsaicin ($1\text{--}3\ \mu\text{g kg}^{-1}$) and 5-HT ($3\text{--}15\ \mu\text{g kg}^{-1}$) were administered repeatedly at 10 min intervals until the response was stable. Bradykinin (BK, $3\ \mu\text{g kg}^{-1}$) and substance P ($10\ \mu\text{g kg}^{-1}$) were injected only twice, before and after the administration of the test drugs, at a 30 min interval. BK ($0.5\ \mu\text{g kg}^{-1}$) was also tested in the presence of propranolol ($1\ \text{mg kg}^{-1}$ i.p. just before the anaesthetic, plus $1\ \text{mg kg}^{-1}$ i.v. 20 min before the first challenge with BK). The test drugs or the vehicle were injected intravenously 2 min before the bronchoconstrictor challenge. Each animal received only a single administration of vehicle or drug. Percentage changes in the response to bronchoconstrictor agents (post-treatment versus pretreatment increase in PIP) were calculated. Changes in drug-treated groups were compared with those in vehicle-treated animals by means of Student's unpaired *t* test. A *P* value of 0.05 or less was taken to be significant.

In another experimental series the dose-response (increase in PIP) curves to substance P and 5-HT were evaluated in the presence and absence of enalaprilic acid, $200\ \mu\text{g kg}^{-1}$ i.v. Substance P was injected at increasing doses ($1\text{--}30\ \mu\text{g kg}^{-1}$), at intervals of 5 min between successive administrations. After 1 h the dose-response curve was repeated, 2 min after administration of vehicle or enalaprilic acid. 5-HT ($1\text{--}30\ \mu\text{g kg}^{-1}$) was injected at 2 min intervals between doses. About 20 min after completion of the first dose-response curve, a second curve was constructed, 2 min after the injection of vehicle or enalaprilic acid. The bronchoconstrictor responses to substance P and 5-HT after the administration of enalaprilic acid were

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compared with those obtained in vehicle-treated animals (Student's unpaired *t* test). In separate groups of animals, the effects of enalaprilic acid on serum and lung ACE activity were evaluated. Enalaprilic acid was used for this purpose, because plasma and tissue ACE inhibition induced by this agent is stable on storage, contrary to that of captopril (Cohen & Kurz, 1983). The animals were prepared as described above. After 20 min of equilibration, vehicle or a single dose of enalaprilic acid ($1.6\text{--}1000\text{ }\mu\text{g kg}^{-1}$) was injected i.v. Between 2 and 3 min after administration, thoracotomy was performed, blood was drawn from the heart and the lungs were excised. Serum and lungs (after perfusion with normal saline through the pulmonary artery) were frozen at -20°C . ACE activity was evaluated by incubating aliquots of serum or lung homogenates with the artificial substrate hippurylglycylglycine in 50 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulphonate) buffer, pH 8, containing 300 mM sodium chloride and 400 mM sodium sulphate (total volume $70\text{ }\mu\text{l}$). After 60 min at 37°C , the reaction was stopped by adding $20\text{ }\mu\text{l}$ 0.5 N perchloric acid and $410\text{ }\mu\text{l}$ distilled water. Enzymatically released hippuric acid was extracted into $500\text{ }\mu\text{l}$ ethyl acetate, $100\text{ }\mu\text{l}$ of which were brought to dryness in a stream of air, at 37°C . Residues were dissolved in $500\text{ }\mu\text{l}$ mobile phase (see below) and analysed by h.p.l.c. on a reverse phase column (Nucleosil C_{18} , $5\text{ }\mu\text{m}$, $15 \times 0.46\text{ cm}$, HPLC Technology) connected to a model 6000 A pump (Waters-Millipore) through a model U6K injector (Waters-Millipore). Peaks eluted isocratically with 20% acetonitrile in 50 mM potassium phosphate pH 3 (flow 1 ml min^{-1} , pressure 2000 psi ca.) were detected at 228 nm by means of a LC-85B spectrophotometric detector (Perkin-Elmer). Parallel analysis of standards revealed an overall 75% recovery of added hippuric acid. Activity was calculated as units (μmol hippuric acid formed per min) per ml (g) of serum (fresh tissue) at 37°C . Percentage inhibition of serum and lung ACE in drug-treated animals when compared to controls was calculated. ED_{50} s and 95% confidence limits were calculated by means of linear regression analysis of the dose-response curves.

Drugs used were: ethyl urethane (Fluka), gallamine triethiodide, ($\pm$)-propranolol hydrochloride, 5-hydroxytryptamine creatinine sulphate and bradykinin triacetate (Sigma), capsaicin (Serva), substance P (kindly donated by Dr P. Rovero, Menarini), prazosin hydrochloride (Pfizer), captopril (Squibb) and enalaprilic acid (Merck, Sharp & Dohme).

Results

Effects of bronchoconstrictor agents

Dose-response curves were constructed for the four bronchoconstrictor agents. Neither the maximal responses (ranging between 40 and $50\text{ cmH}_2\text{O}$) nor the slopes of the curves were significantly different, as estimated by analysis of variance (data not shown). The doses of the agonists employed in the subsequent experiments and their bronchoconstrictor effects, before and after vehicle administration, are shown in Figure 1. The aim was to obtain a BC of similar magnitude with all four agonists, in order to compare the effects of ACE inhibitors on the different responses. This aim was mainly achieved with substance P, capsaicin and bradykinin, since the BC induced by these agents was similar ($P > 0.05$ by analysis of variance). On the other hand, the BC obtained with 5-HT was definitely more intense. This is due mainly to the fact that the response to a fixed dose of 5-HT tended to increase slowly: thus, when the response was stabilized, usually after 4–6 administrations, the BC was generally increased to a considerable extent with respect to the first response. As also shown in Figure 1, the second response to substance P was slightly, but significantly reduced, while no significant difference was found between the increases in PIP evoked by the two successive challenges with capsaicin, 5-HT and BK. In contrast, in the presence of propranolol, the second response to BK was only 60% of the first

Figure 1 Effects of vehicle administration on the bronchoconstriction (increase in pulmonary inflation pressure, PIP) induced by substance P ($10\text{ }\mu\text{g kg}^{-1}$), capsaicin ($1\text{--}3\text{ }\mu\text{g kg}^{-1}$), 5-hydroxytryptamine (5-HT, $3\text{--}15\text{ }\mu\text{g kg}^{-1}$) and bradykinin (BK, $3\text{ }\mu\text{g kg}^{-1}$) in (a) anaesthetized guinea-pigs and by BK ($0.5\text{ }\mu\text{g kg}^{-1}$) in (b) anaesthetized guinea-pigs pretreated with propranolol. The first column (stippled) represents the response observed before administration of the vehicle; the second column (solid) represents the response observed two min after vehicle administration. Mean values are presented and bars show s.e.mean ($n = 5\text{--}10$). * $P < 0.05$ by Student's paired *t* test.

response, suggesting that under these experimental conditions the airways exhibit tachyphylaxis to BK.

Effects of captopril

Generally speaking, captopril produced an increase in the amplitude of the majority of the bronchoconstrictor responses, while their onset rate and duration were not apparently affected, as seen in Figure 2. Therefore, we always refer to changes in the amplitude of the response, if not otherwise stated.

Captopril potentiated dose-dependently the response to substance P (Figure 3a) and capsaicin (Figure 3b). In the case

Figure 2 Representative tracings showing the effects of captopril ($200\text{ }\mu\text{g kg}^{-1}$ i.v.), administered at the arrow, on the bronchoconstriction evoked by substance P ($10\text{ }\mu\text{g kg}^{-1}$) (a), capsaicin ($3\text{ }\mu\text{g kg}^{-1}$) (b), bradykinin (c: $3\text{ }\mu\text{g kg}^{-1}$, in the absence of propranolol; d: $0.5\text{ }\mu\text{g kg}^{-1}$, in the presence of propranolol) and 5-hydroxytryptamine ($10\text{ }\mu\text{g kg}^{-1}$) (e) in the anaesthetized guinea-pig. Capsaicin and 5-hydroxytryptamine were administered repeatedly at 10 min intervals, until a stable response was obtained, while substance P and bradykinin were injected only twice, at an interval of 30 min. Captopril was given 2 min before the bronchoconstrictor agents.

Figure 3 Effects of captopril (●) and enalaprilic acid (○) on the bronchoconstriction induced by (a) substance P ($10 \mu\text{g kg}^{-1}$), (b) capsaicin ($1\text{--}3 \mu\text{g kg}^{-1}$), (c) bradykinin ($3 \mu\text{g kg}^{-1}$, in the absence (1) and $0.5 \mu\text{g kg}^{-1}$ in the presence (2) of propranolol) and (d) 5-hydroxytryptamine ($3\text{--}15 \mu\text{g kg}^{-1}$) in the anaesthetized guinea-pig. The test drugs were injected intravenously 2 min before the bronchoconstrictor challenge. Results are expressed as % increase over control responses, shown in Figure 2. Mean values are presented with vertical lines showing s.e.mean ($n = 4\text{--}10$). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ by Student's unpaired *t* test.

of substance P, the threshold effective dose was $1.6 \mu\text{g kg}^{-1}$ and maximal effect was a 442% increase over control response; in the case of capsaicin, the threshold dose was $40 \mu\text{g kg}^{-1}$ and maximal effect was 92%. 5-HT was potentiated significantly (38%) by captopril only at the dose of $1000 \mu\text{g kg}^{-1}$ (Figure 3d). On the other hand, captopril $200\text{--}1000 \mu\text{g kg}^{-1}$ did not potentiate BK in the absence of propranolol, while at $200 \mu\text{g kg}^{-1}$ it did potentiate this kinin (207%) in β -blocked animals (Figure 3c).

Effects of enalaprilic acid

The effects of enalaprilic acid on substance P- and 5-HT-induced BC, shown in Figure 3a and d respectively, are essentially similar to those of captopril. Enalaprilic acid was assayed also for its ability to inhibit serum and lung ACE under the same experimental conditions used for the BC experiments (Figure 4a): a dose-dependent inhibition of ACE was found at doses of $1.6\text{--}1000 \mu\text{g kg}^{-1}$ in both tissues. The ED_{50} s and 95% confidence limits were 7.6 ($3.6\text{--}15.5$) $\mu\text{g kg}^{-1}$ for serum and 9.4 ($2.5\text{--}31.5$) $\mu\text{g kg}^{-1}$ for lung ACE inhibition. As seen in Figure 4b, substance P potentiation appeared well correlated to ACE inhibition, although this correlation was significant only for serum ACE.

In a separate group of animals the effects of enalaprilic acid, $200 \mu\text{g kg}^{-1}$, on the dose-response curves to substance P and 5-HT were evaluated. Substance P was markedly potentiated along the whole dose-response curve (Figures 5 and 6b).

In this case, time-integrated changes in PIP (between 0 and 5 min) were affected more intensely than the maximal amplitude of BC, since the duration of the response was increased in a more substantial manner than the BC peak (Figures 5 and 6b). On the other hand, enalaprilic acid produced a slight potentiation of 5-HT: a significant effect on either BC peak or time-integrated change in PIP was observed only at a dose of $10 \mu\text{g kg}^{-1}$ of the agonist (36%, $P < 0.05$).

Hypotensive effects of ACE inhibitors and effects of prazosin on bronchoconstriction

Both captopril and enalaprilic acid produced a dose-dependent hypotensive action in the anaesthetized guinea-pig,

Figure 4 Effects of enalaprilic acid on serum (○) and lung (●) angiotensin converting enzyme (ACE) activity (a) and correlation between this ACE-inhibitory activity and potentiation of substance P-induced bronchoconstriction (b) in the anaesthetized guinea-pig. The test drug was administered 2–3 min before bleeding and lung excision. Mean values and s.e.mean (vertical lines) are presented ($n = 3$). ACE activity in control guinea-pig was: $6.28 \pm 0.28 \text{ u ml}^{-1}$ (serum) and $8.60 \pm 3.40 \text{ u g}^{-1}$ (lungs). Mean values of ACE inhibition induced by enalaprilic acid were correlated to mean values of substance P potentiation, induced by corresponding doses of the drug, which are shown in Figure 3a. The correlation coefficients were $r = 0.997$ for ACE inhibition in serum ($P < 0.05$) and $r = 0.885$ for ACE inhibition in lungs ($P > 0.05$).

with enalaprilic acid being slightly more potent than captopril (Figure 7). In order to ascertain the influence of hypotension on BC, the effects of prazosin on the response to bronchoconstrictor agents were evaluated. The drug was administered at a dose of $200 \mu\text{g kg}^{-1}$, which caused a $44 \pm 2\%$ ($n = 18$) fall in mean blood pressure, while having no effect on basal PIP. Prazosin caused a slight but significant ($P < 0.05$) enhancement of the BC induced by capsaicin ($30 \pm 8\%$, $n = 8$) and 5-HT ($32 \pm 8\%$, $n = 6$), but did not affect the BC induced by substance P ($-5 \pm 9\%$, $n = 4$).

Figure 5 Representative tracing showing the effects of enalaprilic acid, administered at the arrow ($200 \mu\text{g kg}^{-1}$), on the bronchoconstriction induced by increasing doses of substance P, administered at the dots, in the anaesthetized guinea-pig. About one hour after completion of the first dose-response curve, the second curve was constructed, 2 min after the administration of enalaprilic acid.

Figure 6 Bronchoconstriction induced by 5-hydroxytryptamine and substance P and effects of enalaprilic acid ($200 \mu\text{g kg}^{-1}$). (a) Dose-response curves for 5-hydroxytryptamine ($\square$) and substance P ($\blacksquare$). (b) Open and closed columns represent enalaprilic acid-induced increases of maximal amplitude of bronchoconstriction at different doses of 5-hydroxytryptamine and substance P, respectively. Hatched columns represent increases of time-integrated (between 0 and 5 min) changes in pulmonary inflation pressure induced by substance P. Results are shown as % changes over response observed in vehicle-treated animals, shown in (a). Mean values and s.e.mean (vertical lines) are presented. * $P < 0.05$; ** $P < 0.01$ by Student's unpaired t test.

Discussion

The present experiments were designed to permit a direct comparison of the effects of ACE inhibitors on the BC induced by different agonists. The doses of substance P, capsaicin and BK used in the main experiments were broadly equipotent in producing BC, thus allowing the comparison of potentiation expressed as % increase over control response. On the other hand, with 5-HT we obtained a BC which was more intense than that observed with the other agents, but still lying on the lower part of the dose-response curve. Thus, a comparison of the potentiation of 5-HT with those of the other agonists may not be appropriate. However, we have shown in separate experiments that potentiation of 5-HT by enalaprilic acid is similarly slight along the whole dose-response curve of the agonist (Figure 6b).

Our results show that ACE inhibitors potentiate the bronchomotor response induced by the four agents tested, but the potency and efficacy of this enhancing action appears different in each case and BK is potentiated only in the presence of

Figure 7 Hypotensive effects of captopril ($\bullet$) and enalaprilic acid ($\circ$) in anaesthetized guinea-pigs. Each animal received a single dose of one agent. Results are expressed as % decrease of basal mean blood pressure.

Mean values and s.e.mean (vertical lines) are presented ($n = 3-5$). Basal mean blood pressure was 36 ± 1.3 mmHg ($n = 30$).

propranolol. Moreover, ACE inhibitors cause a profound hypotension in the anaesthetized guinea-pig, as already demonstrated with captopril by Greenberg *et al.* (1979), but in contrast with the effects observed in other normotensive animals, such as rats, rabbits, cats and monkeys (Rubin *et al.*, 1978).

In order to check the possible role of hypotension in the enhancement of BC, we examined the effects of prazosin: this drug slightly potentiated capsaicin- and 5-HT-induced BC, but did not appear to affect substance P-induced BC.

Therefore hypotension *per se* is likely to have a slight potentiating effect on BC, although we do not have an explanation for this finding at the moment. As a consequence, the potentiating effects of ACE inhibitors may have been slightly overestimated and it is possible that potentiation of 5-HT, which was always less than 40%, is due mainly to the hypotensive action of these drugs. However, the same explanation cannot be valid for capsaicin, which was potentiated by captopril to a significantly larger extent ($P < 0.05$ vs 5-HT), and for substance P, which was not potentiated by prazosin in our experiments, while a striking potentiation was produced by ACE inhibitors. The latter effect appears well correlated to serum and lung ACE inhibition. In fact, substance P can be metabolized by ACE (Cascieri *et al.*, 1984), as well as by other enzymes from different sources, such as serine proteinases (Hanson & Lovenberg, 1980), acetylcholinesterase (Chubb *et al.*, 1980) and enkephalinase (Skidgel *et al.*, 1984). The results of these experiments indicate that ACE plays a prominent role in the degradation of substance P itself and/or of another mediator subserving the actions of substance P, since an inhibition of 50% or less of serum and lung ACE is sufficient to produce a massive increase of substance P-induced BC. A similar mechanism, e.g. inhibition of the metabolism of substance P released from sensory nerves, can also explain the potentiation of capsaicin, but in this case the effects of captopril are inferior both in potency and efficacy. We can think of two possible explanations for this difference: (a) capsaicin can release a number of neuropeptides (including calcitonin gene-related peptide and neurokinin A, which have been identified in the airways and contract airway smooth muscle), as well as substance P (Barnes, 1987). These neuropeptides are not metabolized by ACE, and consequently are not potentiated by captopril; (b) Substance P is probably released in extravascular sites where not only ACE, but also other enzymes, e.g. enkephalinase, play a physiological role (Borson *et al.*, 1987). ACE located in these sites may be less readily accessible to inhibitors than the enzyme located at the endothelial luminal surface, where exogenous substance P is most probably degraded. The first hypothesis may provide an explanation for the lower efficacy of captopril against capsaicin, while the second hypothesis might also explain the lower potency of the drug, due to limited availability at the active site.

Since ACE is identical with kininase II, potentiation of BK

was obviously expected: it is thus a paradox that BK is the only bronchoconstrictor which was not potentiated to any extent in our experiments. The doses of captopril we have tested ($200\text{--}1000\text{ }\mu\text{g kg}^{-1}$) are well above those showing ACE-inhibitory activity, thus definitely inhibiting the degradation of BK. However, our results are in agreement with those of Greenberg *et al.* (1979), who showed that captopril is capable of enhancing the bronchomotor effects of BK in the presence but not in the absence of propranolol. These authors suggest that captopril causes an increase of both BC and release of adrenomedullary catecholamines induced by BK, with the latter effect antagonizing the former one. Thus, the overall effect of captopril on BK-induced BC is null, unless the animals are β -blocked. In this context, it is well known that an adrenergic bronchodilator reflex mechanism also occurs with other agonists (McCulloch *et al.*, 1967) and therefore it would be interesting to investigate the effects of β -blockade on the

interaction between substance P or capsaicin and ACE inhibition. Anyhow, this adrenergic reflex is likely to be less important in these cases, since the latter agonists also appear potentiated in the absence of β -blockade.

In conclusion, ACE inhibitors appear capable of enhancing the bronchomotor response to substance P and capsaicin. These results suggest that ACE plays an important role in modulating the *in vivo* response to exogenously administered or endogenously released substance P, and may offer a mechanistic explanation of the cough and bronchial hyperreactivity observed in patients receiving treatment with ACE inhibitors.

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