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Effect of angiotensin-converting enzyme (ACE) inhibitors on ciliary activity

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Abstract

The effect of three angiotensin-converting enzyme (ACE) inhibitors on ciliary beat frequency was monitored with a view to investigating whether ciliotoxicity could account for the high incidence of cough associated with the use of those agents. An additional objective was to investigate whether intranasal administration of those ACE inhibitors was safe. It was found that captopril and enalapril maleate reduced ciliary beat frequency in a dose-dependent manner. The effect of captopril was reversible while that of enalapril maleate was not. Lisinopril was non-ciliotoxic at four concentrations up to 113 mM.

Key words: Angiotensin-converting enzyme; Enzyme inhibitor; Captopril; Enalapril; Lisinopril; Cilia

1. Introduction

Angiotensin-converting enzyme (ACE) inhibitors are widely prescribed as anti-hypertensive agents and in heart failure. Indeed, there is considerable expert opinion supporting their use as first-line agents in the management of high blood pressure. However, a common side-effect which mars their use is a dry, persistent and irritating cough which interferes with sleep and/or speech (Havelka, 1982; Webb et al., 1986; Coulter and Edwards, 1987).

The mechanism by which the ACE inhibitors induce coughing is still unclear. ACE is a membrane-bound peptidase which is known to be predominantly present in lung tissue (Swerts et al., 1979; Johnson et al., 1985). While its precise location in the trachea is still unknown, ACE activity is present (Dusser et al., 1988). Inhibition of ACE results in accumulation of bradykinin and substance P which may stimulate cough receptors directly (Yeo et al., 1991). Moreover, bradykinin enhances prostaglandin production, including PGE_2, which induces coughing (Do et al., 1990).

Irrespective of the underlying biochemical mechanism, it is possible that ACE inhibitors impair mucociliary clearance or ciliary activity. This leads to local accumulation of irritants and hence the induction of cough. This study was

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Abbreviations: ACE, angiotensin-converting enzyme; LR, Locke-Ringer; CBF, ciliary beat frequency; PGE_2, prostaglandin E_2.

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initiated to test this hypothesis by investigating the effect of clinically used ACE inhibitors on ciliary beat frequency using rat tracheal cilia as an in vitro model.

An increasing number of peptide drugs are being introduced into therapeutics. To circumvent problems associated with poor oral bioavailability, the nasal route is being actively investigated for the systemic delivery of such drugs (Zhou and Li Wan Po, 1991). Indeed, a number of therapeutic peptides such as salcalcitonin and vasopressin are already successfully administered intranasally. Therefore, an added objective was to investigate whether the ACE inhibitors, as models of peptide drugs, could be administered intranasally without adverse effects on cilia.

2. Materials and methods

Captopril, enalapril maleate and lisinopril were gifts from Squibb & Sons (Moreton, Merseyside, U.K.), Merck & Co. Inc. (Endfield, Middlesex, U.K.) and Imperial Chemical Industries PLC (Macclesfield, Cheshire, U.K.), respectively.

Tracheal rings were prepared using tracheas removed from freshly killed adult male Wistar rats weighing 300–400 g. Each trachea was cut into rings about 0.5 mm thick with the ciliated epithelium still intact. The rings were maintained in 5 ml Locke-Ringer (LR) solution for at least 30 min, at 37°C prior to use to stabilise ciliary beats.

The ciliated tracheal rings were examined using an inverted Olympus (CK2-TRP) binocular microscope equipped with a compact video camera (Hitachi KP-143) connected to a Hitachi video monitor. Ciliary movement was recorded on an Akai (model 425-EK) video cassette recorder (VCR) for later analysis. The preparations were examined using a $\times 20$ lens and a $\times 10$ eyepiece.

The signal produced by the beating cilia was picked up by a photo-sensitive probe from the video monitor and was converted by fast Fourier transform into a frequency spectrum, using an Opus PC III microcomputer with on-board A/D converter. In the present study, the ciliary beat frequency (CBF) was measured every 30 s for up to 2 h.

Each tracheal ring was observed at three different sites. Hence, mean values of CBF were from nine different areas in three individual rats. After measurement of the control ciliary beat frequency, the control solution (LR) was replaced by LR solution containing the various drugs. In between test solutions, the chamber was rinsed several times with LR control solution.

Dose-response studies were carried out with the three angiotensin-converting enzyme (ACE) inhibitors, captopril, enalapril maleate and lisinopril, by serial dilution of a suitable stock solution using LR as diluent.

3. Statistics for quantifying ciliotoxicity

When the cilia are exposed to toxic substances, the ciliary beat frequency (CBF) slows down relative to the initial values. Therefore, when the CBF is continuously monitored, damage to the cilia can be seen as a CBF-time decay curve. The more toxic the compound, the sharper is the decline in CBF with time (Van de Donk et al., 1982). Early studies used CBF measurements at specific time points for comparative purposes such as when comparing the toxicities of various compounds. In more recent literature, CBF-time curves are displayed for such purposes (Tamaoki et al., 1989). In a recent report we advocated the wider use of area under the curve when comparing drug responses over time (Chan and Li Wan Po, 1992) in line with the growing recognition that summary statistics should be used when appropriate (Matthews et al., 1990). In this study the area under the curve is the area between the CBF = 100% and the CBF-time curve. We refer to this area as the complement of the area under the curve (CAUC), since in most other kinetic studies the area under the curve is the area between the abscissa and the relevant curve. The area under the curve was calculated using the trapezoidal rule and a Lotus 1-2-3® Macro (Chan and Li Wan Po, 1992).

4. Results

Fig. 1 and 2 show the ciliary beat frequency decay profiles for cilia exposed to various concen-

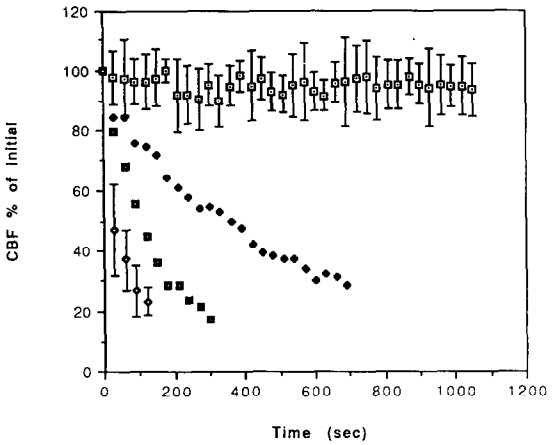

Fig. 1. Ciliary beat frequency decay for cilia exposed to various concentrations of captopril. (⊡) Control; (◆) 4.56 mM; (■) 6.84 mM; (◆) 9.1 mM. The errors bars refer to standard error ($n = 9$).

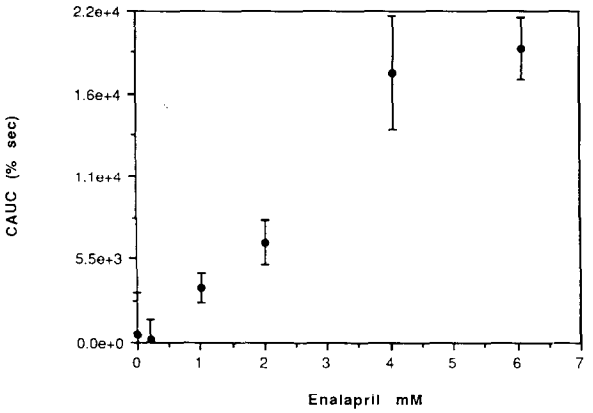

Fig. 3. Ciliotoxicity of enalapril in rat trachea. See text for definition of CAUC. The error bars refer to standard error ($n = 9$).

trations of captopril and enalapril maleate, respectively. For clarity the standard error of the CBF is shown for only the control and the highest concentrations of the drugs used. The variances of the CBF are of the same magnitude at all the different concentrations used. Lisinopril induces relatively little reduction in ciliary beat frequency at concentrations of up to 133 mM.

Both captopril and enalapril maleate show a dose-response relationship which was evident in the range of concentrations used in the present study. Fig. 3 plots the profile for enalapril while Fig. 4 shows the corresponding profile for captopril. The areas under the curve were calculated from time zero to the shortest observation time used in the studies in order to ensure comparable values. In other words, the area under the curve corresponded to the time of observation for the most toxic solution used in the study. Removal of the drug solution and its replacement by fresh Locke-Ringer solution led to recovery in beat by

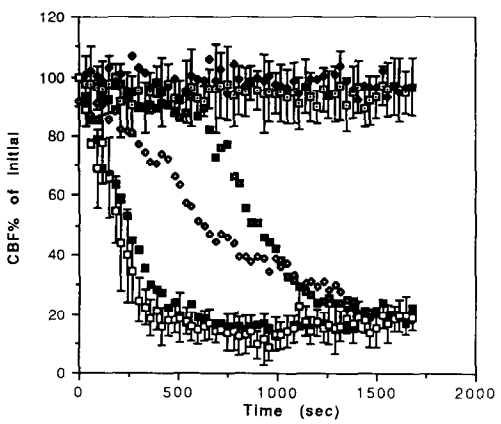

Fig. 2. Ciliary beat frequency decay for cilia exposed to various concentrations of enalapril. (⊡) Control; (◆) 0.2 mM; (■) 1.02 mM; (◆) 2.03 mM; (■) 4.06 mM; (□) 6.09 mM enalapril.

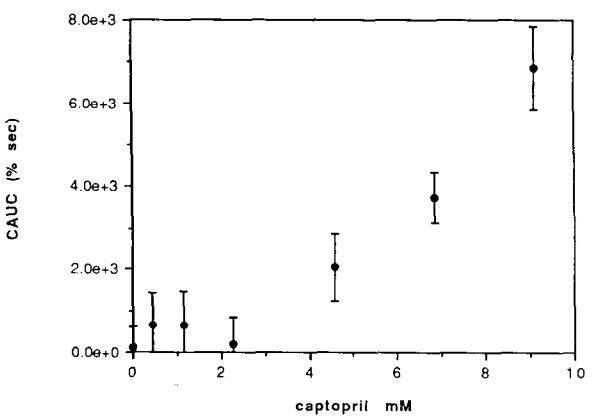

Fig. 4. Ciliotoxicity of captopril in rat trachea. See text for definition of CAUC.

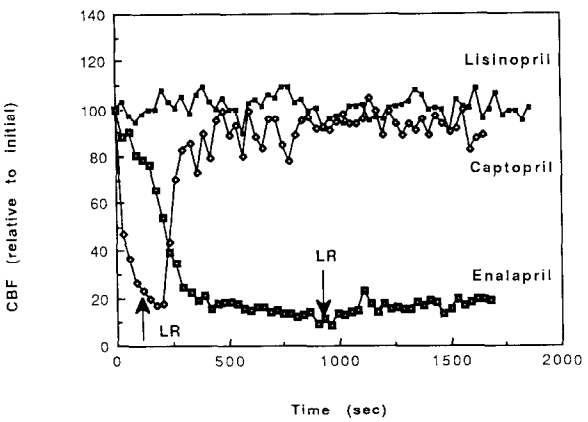

Fig. 5. Time vs CBF plot for captopril (9.1 mM), enalapril (6.09 mM) and lisinopril (6.8 mM). LR, Locke-Ringer solution.

cilia exposed to captopril but not to enalapril (Fig. 5).

5. Discussion

The results suggest that ACE inhibitors inhibit ciliary beat. The resulting states may in turn lead to coughing but several observations justify further comment. Lisinopril produced little inhibition of ciliary beat. However, it is also known to induce cough in clinical use (Woo and Chan, 1991; Os et al., 1992). Lisinopril is reported to be slow acting (Thomas Morson Pharmaceuticals, 1990–1991) and it may well be that the exposure time used in our model is insufficient to detect any changes induced in ciliary beat. However, there are no comparative data on the onset of cough following initiation of treatment with lisinopril, enalapril and captopril.

The mechanism by which ACE inhibitors induce coughing is still unknown. Patients receiving ACE inhibitors show increased sensitivity to capsaicin-induced cough (Fuller and Choudry, 1987). Therefore, hypersensitisation to normally subthreshold stimuli of the cough reflex is a possible mechanism. Angiotensin-converting enzyme inactivates bradykinin and substance P so that patients on ACE inhibitors are expected to show higher resting levels of those mediators which may induce coughing (Do et al., 1990). Moreover, bradykinin enhances production of prostaglandin (PGE_2) which is tussogenic (Do et al., 1990).

Whether the ciliotoxic effects observed with enalapril and captopril share any of those biochemical mechanisms is not known. An investigation of the direct effects of the ACE inhibitors, however, does not adequately test these possibilities and extension of the present work is worthwhile. Interestingly, using rabbit cultured tracheal epithelium, Kobayashi et al. (1990) reported that angiotensin II stimulated ciliary beat frequency in a dose-dependent manner in the concentration range 10^{-6}–10^{-13} M. The effect observed in our study with ACE inhibitors is therefore consistent with their results.

The reversible ciliotoxic effects observed with captopril but not enalapril are surprising. It is possible that the higher lipophilicity conferred to the enalapril molecule by esterification of enalaprilat leads to rapid partitioning into the lipoidal membranes. Once this has taken place flushing out with fresh medium becomes more difficult.

ACE inhibitor induced coughing is a perplexing problem affecting many patients receiving them. Therapeutic approaches evaluated for controlling it include prostaglandin-synthetase inhibitors and sodium cromoglycate. Until the mechanism for its induction becomes clearer, therapy is likely to remain largely empirical and relatively ineffective.

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