ANGIOTENSIN-CONVERTING ENZYME INHIBITORS SUPPRESS HYDROXYL RADICAL GENERATION INDUCED BY NONYLPHENOL IN STRIATUM

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Introduction

Much of the concern appears focused on environmental chemicals such as para-nonylphenol which disrupt various tissues via steroid receptor. Environmental chemicals with estrogenic activity are considered to cause a variety of adverse effects such as reproductive disorders, endocrine disorders, and a variety of cancers[1, 2].

Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is oxidized by monoamine oxidase and converted to 1-methyl-4 phenylpyridinium ion (MPP^+). MPTP produces a parkinsonian syndrome after its conversion to MPP^+ [3, 4]. The etiology of Parkinson's disease remain obscure. The cytotoxic hydroxyl radical has been implicated in dopamine neurotoxicity caused by MPTP and iron[5]. Oxidative stress may be involved in the pathogenesis of idiopathic Parkinson's disease in the nigra.

Angiotensin-converting enzyme (ACE) is widely distributed in the brain. In the basal ganglia angiotensin-converting enzyme is associated with neurons in the striatum. Although the role of angiotensin-converting enzyme inhibitors in free radical-scavenging effects are still speculative [6], the activity of captopril is believed to be due to the presence of an SH-group in its structure [7]. However, non-SH containing angiotensin-converting enzyme inhibitors also provide protection against free radical-induced injury[8].

We recently reported that para-nonylphenol induces hydroxyl radical formation in rat striatum[9]. In this study we examined the antioxidant effects of angiotensin-converting enzyme inhibitors on para-nonylphenol- and MPP^+ (1-methyl-4-phenylpyridinium ion)-induced hydroxyl radical formation and dopamine efflux in extracellular fluid of rat striatum, using a microdialysis technique.

Materials and Methods

Animals. Adult male Wistar rats (300-400 g) were housed in an environmentally controlled room (20-25°C, 50-60% of humidity) with food and water available ad libitum for 4 days prior to the experiments. The animals were anesthetized with chloral hydrate (400 mg/kg i.p.; Sigma Chemical Co., St. Louis, MO) and prepared for inntracranial microdialysis brain perfusion. This study was approved by the Ethics Committee for Animal Experiments, Oita Medical University.

Experimental protocol. MPP+ was purchased from Research Biochemicals Inc. MA. Captopril and enalaprilat were provided purchased from Sigma. A guide cannula was implanted stereotaxically on top of the caudate nucleus (stereotaxic coordinates: AP: 1.0, R/L: 2.5, H: -7 mm from dura matter) [10]. In preliminary experiments, the recovery rate of 10^{-7} M dopamine was about 21 % at a flow rate of 1 µl/min. The drugs were dissolved in Ringer's solution containing 147 mM NaCl, 2.3 mM $CaCl_2$ and 4 mM KCl, pH 7.0 for perfusion (1 µl/min) through a microdialysis

probe into the striatum. Following the scheduled 60-min washout with Ringer's solution, MPP^+ (5 mM or 5 nmol/μl/min) was infused for 15 min (total dose: 75 nmol) to evoke the sustained, voltage-regulated, and calcium-dependent release of dopamine. The striatum was then perfused with Ringer's solution (1 μl/min) for at least 60 min.

Analytical procedures. The dialysate samples were immediately injected for hydroxylradical and dopamine analysis into an HPLC-EC equipped with a glassy carbon working electrode (EICOM CORP., Kyoto, Japan) and an analytical reverse-phase Eicompak MA-5 ODS column (5 μm, 4.6x 150 mm; EICOM). The working electrode was set at a detector potential of 0.75 V. Each liter of the mobile phase contained 1.5 g heptane sulfonic acid sodium salt (Sigma), 0.1 g Na_2EDTA, 3 ml triethylamine (Wako) and 125 ml acetonitrile (Wako) dissolved in H_2O.

Statistical analysis. All values are presented as means ± S.E.M. The significance of differences was determined by using an analysis of variance (ANOVA) with Fisher's post hoc test. A P value of less than 0.05 was regarded as being statistically significant.

Results and Discussion

We first examined whether para-nonylphenol enhanced hydroxylradical formation and dopamine efflux induced by MPP^+. As shown in Figures 1 and 2, para-nonylphenol (10 μM) enhanced dopamine efflux and hydroxylradical formation trapped as DHBA induced by 5 mM MPP^+.

We compared the ability of non-SH-containing angiotensin-converting enzyme inhibitor (enalaprilat) with a SH-containing angiotensin converting enzyme inhibitor (captopril) to scavenge hydroxylradicals and dopamine efflux. When 100 μM enalaprilat or captopril was infused in para-nonylphenol and MPP^+-treated rats, the formation of dopamine and hydroxyl radical formation was significantly decreased, as compared with that in the para-nonylphenol and MPP^+-treated control (Figs.1 and 2). Both inhibitors were able to scavenge hydroxylradicals and dopamine efflux induced by para-nonylphenol and MPP^+.

To further investigate whether the suppressive effect of captopril and enalaprilat on para-nonylphenol and MPP^+-induced hydroxyl radical formation was based on Fenton-type reaction, the hydroxylradical formation was measured in para-nonylphenol and MPP^+-treated rats in the presence of iron. When iron (II) (2, 5 and 10 μM) was administered to 10 μM para-nonylphenol and 5 mM MPP^+-treated animals, iron (II) clearly produced a dose-dependent increase in the levels of DHBA, showing a positive linear correlation between iron (II) and hydroxyl radical formation trapped as DHBA ($R^2 = 0.98$) in the dialysate (Figure not shown). When corresponding experiments were performed in the presence of captopril or enalaprilat, similar results were observed. However, the levels of DHBA in ACE inhibitor-treated group were significantly lower than that observed in para-nonylphenol and MPP^+-treated group ($p<0.05$). The results suggest that the suppressive effect of ACE inhibitors on hydroxyl radical formation was based on Fenton-type reaction.

In summary, the results in the present study suggest that angiotensin-converting enzyme inhibitors may protect against para-nonylphenol and MPP+-induced hydroxyl radical formation via suppressing dopamine efflux in the rat striatum.

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Fig.1 Effect of ACE inhibitors on dopamine efflux

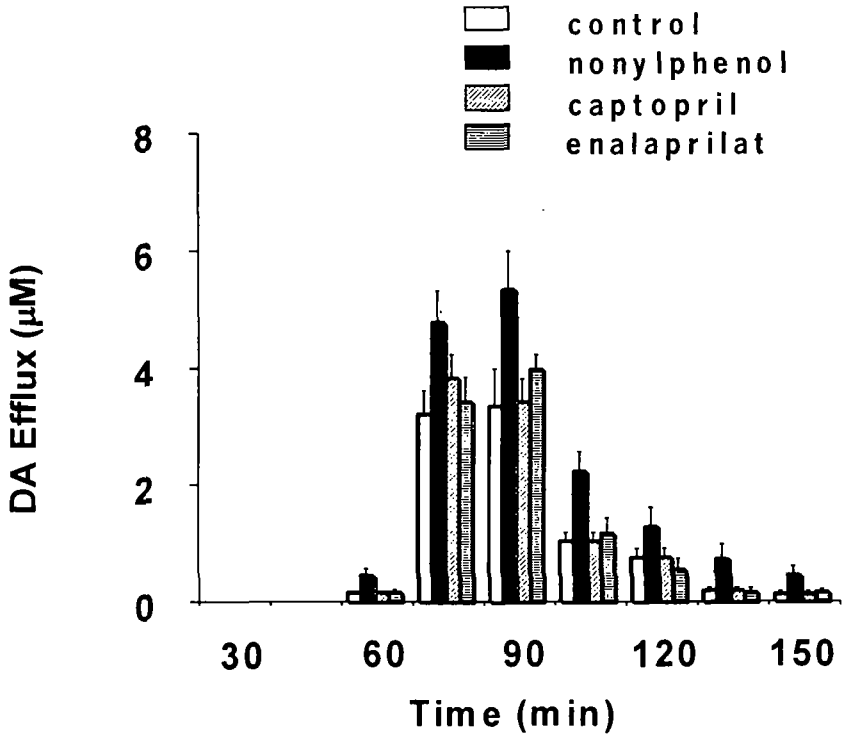

Fig.2 Effect of ACE inhibitors on DHBA formation