Antioxidant effects on prostaglandin synthesis in rabbit kidney medulla slices

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Summary. Sodium diethyldithiocarbamate, 2,6-di-tert-butylphenol and N,N′-diphenyl-p-phenylenediamine inhibited the generation of medullary prostaglandin E, but 1,2-dimethoxyethane (OH· scavenger) and 2,5-dimethylfuran ($^1O_2$ scavenger) stimulated basal prostaglandin E production 1.2–1.3-fold. These results suggest that the balance between formation and removal of cellular lipid peroxides sets a peroxide level that can regulate the rate of prostaglandin formation in cells.

It has been reported that prostaglandin cyclooxygenase is irreversibly self-deactivated during the oxygenation of arachidonic acid[1]. Egan et al.[2] have shown that prostaglandin cyclooxygenase is irreversibly self-deactivated due to the natural reduction of the hydroperoxide at carbon 15 of prostaglandin G_2 to the hydroxyl on prostaglandin H_2, and during this reduction, radicals, possibly hydroxyl radicals, are formed which could be oxidizing the enzyme. On the other hand, inhibition of cyclooxygenase by glutathione peroxidase[1,3] provided the initial evidence that cyclooxygenase catalysis requires some peroxide which apparently could be generated by cyclooxygenase itself[4]. Recently, we have reported that lipid peroxidation induced by ascorbic acid and Fe^{2+} inhibits medullary generation of prostaglandin E[5].

This paper deals with the effects of several classical antioxidants on the in vitro production of prostaglandin E, in order to investigate the possible involvement of lipid peroxides in regulation of prostaglandin synthesis.

Materials and methods. Male rabbits (2–2.5 kg) were used in the present study. The kidneys were removed from anesthetized (sodium pentobarbital, 30 mg/kg) rabbits and rapidly chilled in ice-cold saline. The kidney medulla was cut into slices (about 1 mm in thickness) with a razor blade on an ice-cold petri dish. In all experiments, rabbit kidney medulla slices (0.4 g) were preincubated in 4.0 ml 0.15 M KCl/0.02 M Tris-HCl buffer (pH 7.4) at 4 °C for 5 min. Following preincubation, the medium was discarded, the slices rinsed twice with the Tris-HCl buffer and incubated with various concentration of drugs at 37 °C for 30 min. After incubation the medium was assayed for prostaglandin E content by a high-performance liquid chromatography method as described in our previous paper[5]. Briefly, prostaglandin E extracted with ethyl acetate (approximately pH 3) was measured after its base-catalyzed conversion to prostaglandin B[6]. Peak heights were measured for the quantification of the extracted prostaglandin B relative to a prostaglandin B_2 standard prepared from authentic prostaglandin E_2.

N,N′-diphenyl-p-phenylenediamine (DPPD) was dissolved in 99.5% ethanol and added to the reaction mixture before initiation. Final concentration of ethanol in the reaction mixture was less than 0.05%, which did not affect prostaglandin E production significantly.

The values presented in this paper are the mean ± standard error. Statistical significance was calculated using Student's paired t-test.

Results and discussion. Lipid peroxides are produced during the enzymatic conversion of arachidonic acid to prostaglandins, thromboxane, prostacyclin and leukotrienes. These peroxides include hydroperoxides of arachidonic acid formed by lipoxygenase and the prostaglandin endoperoxide intermediates produced by the action of prostaglandin endoperoxide synthetase. It can be conceived that a number of steps in the arachidonate-dependent prostaglandin pathway are vulnerable to antioxidant effects.

The effects of antioxidants on the production of prostaglandin E in rabbit kidney medulla slices are shown in the table. The generation of prostaglandin E was markedly inhibited by antioxidants such as sodium diethyldithiocarbamate, 2,6-di-tert-butylphenol and DPPD, but was stimulated slightly by the addition of 1,2-dimethoxyethane as a scavenger of hydroxyl radicals (OH·) or 2,5-dimethylfuran as a scavenger of singlet oxygen ($^1O_2$)[7]. The inhibitory effect of antioxidants was dependent on their concentrations. We reported previously that DPPD inhibited lipid peroxidation induced by ascorbic acid at extremely low concentrations as compared with other antioxidants[8]. These results suggest that the activity of the cyclooxygenase requires lipid peroxide in small quantities, and that free radicals produced during prostaglandin synthesis inhibits prostaglandin cyclooxygenase.

The present study showed that antioxidant could act to reduce peroxide levels and either enhance or inhibit cyclooxygenase activity depending on the type of antioxidant and the concentration. Further studies are in progress.

Effects of various antioxidants on the biosynthesis of prostaglandin E in rabbit kidney medulla slices (n = 5)

Compound	Concentration (M)	Prostaglandin E (μg/g wet wt of tissue)
No addition		2.66 ± 0.19
1,2-Dimethoxyethane	10^{-3}	3.09 ± 0.14
	10^{-4}	3.25 ± 0.23
	10^{-5}	2.71 ± 0.18
2,5-Dimethylfuran	10^{-3}	3.56 ± 0.33
	10^{-4}	3.53 ± 0.47
	10^{-5}	3.48 ± 0.44
Sodium diethyl-dithiocarbamate	10^{-2}	0.56 ± 0.14**
	10^{-3}	0.86 ± 0.23**
2,6-Di-tert-butylphenol	10^{-3}	1.53 ± 0.28*
	10^{-4}	1.98 ± 0.24
N,N′-Diphenyl-p-phenylenediamine	10^{-5}	0.53 ± 0.18**
	10^{-6}	1.06 ± 0.19**

*$0.01 < p < 0.02$; **$p < 0.01$.

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