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THE INHIBITION OF THE FATTY ACID OXYGENASE OF SHEEP VESICULAR GLAND BY ANTIOXIDANTS

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SUMMARY

Antioxidants inhibit the fatty acid oxygenase of sheep vesicular gland and soybean lipoxygenase in an instantaneous, reversible manner. Their inhibitory effectiveness was not related to their traditional antioxidant potencies but seemed to depend on the nature of the enzyme. The destructive effect of eicosa-5,8,11,14-tetraynoic acid, a substrate analog, on vesicular gland oxygenase could be prevented in the presence of either α-naphthol or 2,2,4-trimethyl-6-ethoxy-1,2-dihydroquinoline (Santoquin).

INTRODUCTION

Varied results have been reported for the effect of several antioxidants on prostaglandin formation. Hydroquinone (0.1–0.5 mM)[1–6] and propyl gallate (0.3–0.5 mM)[1,2] stimulated the production of prostaglandin but definite inhibition was observed at greater concentrations. Ascorbic acid[2,5,7] and pyrrogallol[2,4] were less effective in increasing the yield of prostaglandin whereas the process was inhibited by α-tocopherol (0.3–1 mM)[1]. No inhibition was reported for 2,6-di-*tert*-butyl-4-hydroxytoluene at 0.25 mM[8]. In our continuing investigations of the fatty acid oxygenase of sheep vesicular gland, an enzymic component of the prostaglandin biosynthetic pathway[9], we have studied the effect of antioxidants on the oxygenase in order to further elucidate the mechanism of the initial oxygenation process[10,11].

MATERIALS AND METHODS

O_2 absorption measurements were carried out on an automatic multisample continuous recording oxygen electrode system[11] with a 3-ml assay mixture containing 0.1 M Tris–HCl (pH 8.5) buffer, 0.67 mM phenol, 4–40 μM arachidonic acid (Hormel Institute), activated vesicular gland oxygenase (2 mg)[10] and various concentrations of antioxidants (purchased from commercial sources). In some experiments, soybean lipoxygenase was substituted for the above enzyme with concomitant deletion of phenol. In other experiments, eicosa-5,8,11,14-tetraynoic acid (a gift from

Hoffmann-La Roche) and antioxidant were incubated together with vesicular gland oxygenase prior to the addition of arachidonic acid.

RESULTS AND DISCUSSION

A variety of antioxidants were found to inhibit the vesicular gland oxygenase (Table I) at much lower levels than previously reported[1-8], but none of those tested exhibited an additional stimulating effect on the enzyme. Dixon plots[12] for the data indicated that the antioxidants could be subdivided into either competitive or non-competitive inhibitors. The curvature of some plots made the assignment of the nature of the inhibition difficult to determine, and the K_i values assigned to competitive inhibitors may vary by a factor of 2–6. The competitive K_i values from these plots are consistently lower than the corresponding $[I]_{50}$* values determined at 12 μM arachidonic acid although the noncompetitive K_i values agree well with their comparative $[I]_{50}$ values. This is expected since more than the theoretical half saturating amount of competitive inhibitor will be required in the presence of substrate to

TABLE I

INHIBITORY AND OTHER CHARACTERISTICS OF ANTIOXIDANTS

The K_i and $[I]_{50}$ were obtained as described in the text. The values were derived from at least eight determinations. C, competitive inhibitor, N.C., noncompetitive inhibitor. Relative efficiency of the antioxidant is measured by the time in which 20% carotene decomposed in mineral oil at 75 °C.

Antioxidant	K_i *(μM)*	$[I]_{50}$ *(μM)*	*Oxidation potential (V)*	*Relative efficiency*[17]
Santoquin	2 (C)	6.5		
α-Naphthol	4 (N.C.)	3.5	0.68 (ref. 13), 0.933 (ref. 14)	154
NDGA	5 (C)	11	0.782 (ref. 15)	208
BHA	6.5 (N.C.)	6.7	0.64, 0.65 (ref. 13), 1.18 (ref. 15)	130
Guiacol	8 (C)	25		6
β-Naphthol	9 (N.C.)	11	0.70 (ref. 13), 1.153 (ref. 14)	18
Methylene Blue	25 (C)	140		
Propyl gallate	28 (C)	120	0.760 (ref. 15)	165**
Trimethylhydroquinone	37 (N.C.)	56	0.528 (ref. 14)	
Hydroquinone	55 (C)	180	0.56 (pH < 7), 1.23 (pH > 7) (ref. 13) 0.715 (ref. 14)	8
Quercetin	100 (N.C.)	90		
BHT	200 (N.C.)	190	0.57, 0.68 (ref. 13)	142
Ascorbic acid	400 (N.C.)	310	0.40 (ref. 15)	
α-Tocopherol	500 (N.C.)	500	0.754 (ref. 15)	
Citric acid, Na^+	> 1700	> 1700		
Pyrrogallol	N.M.*	N.M.*	0.28 (pH > 7), 0.51 (pH < 7) (ref. 13), 0.676 (ref. 14)	155

* Not measurable because of unusual curvature.

** Ethyl gallate instead of propyl gallate.

Abbreviations: BHA, 2- and 3-*tert*-butylhydroxyanisole; BHT, 2,6-di-*tert*-butyl-4-hydroxytoluene; NDGA, nordihydroguaiaretic acid; Santoquin, 2,2,4-trimethyl-6-ethoxy-1,2-dihydroquinoline; C, competitive inhibitor; N.C., noncompetitive inhibitor.

* $[I]_{50}$ is the concentration of inhibitor that causes 50% inhibition.

TABLE II

COMPARATIVE ANTIOXIDANT INHIBITION OF VESICULAR GLAND OXYGENASE AND SOYBEAN LIPOXYGENASE

Antioxidant	*Vesicular gland dioxygenase* $[I]_{50}$ *(μM)*	*Soybean lipoxygenase* $[I]_{50}$ *(μM)*
α-Naphthol	3.5 (N.C.)	1.5
Trimethylhydroquinone	56 (N.C.)	26
BHT	190 (N.C.)	> 300
Santoquin	6.5 (C)	> 200
Propyl gallate	120 (C)	72
Hydroquinone	180 (C)	22

Abbreviations: see Table I.

reduce the velocity to 50%. All of the antioxidants tested behaved as instantaneous concentration-dependent inhibitors but none showed appreciable time-dependent inhibition of the oxygenase activity[11].

To determine whether these antioxidants exhibited similar effects on soybean lipoxygenase, three competitive and three non-competitive inhibitors were tested (Table II). Two from each category were found to be more effective with lipoxygenase whereas 2,6-di-*tert*-butyl-4-hydroxytoluene and Santoquin (2,2,4-trimethyl-6-ethoxy-1,2-dihydroquinoline) were much less so. An early report by Tappel *et al.*[13] indicated that the relative inhibitory effectiveness of several antioxidants with soybean lipoxygenase was in the following order: nordihydroguaiaretic acid > propyl gallate > catechol ≈ hydroquinone ≈ α-naphthol ≈ α-tocopherol. These differences were much smaller than those that we are describing for the vesicular gland oxygenase. Apparently the inhibitory ability of these agents is influenced by the nature of the

TABLE III

α-NAPHTHOL AND SANTOQUIN PREVENT DESTRUCTIVE ACTION OF EICOSA-5,8,11,14-TETRAYNOIC ACID ON VESICULAR GLAND OXYGENASE

Phenol-activated oxygenase (2 mg) was added to an assay mixture containing 0.66 mM phenol, 6 μM eicosa-5,8,11,14-tetraynoic acid and the indicated concentrations of either α-naphthol or Santoquin at 30 °C. After incubating for *t* min, arachidonic acid (25 μM) was added and O_2 absorption measurements were carried out as previously reported[11]. The decreased activity (%) is calculated as 100 $(1 - v/v_0)$ where v = initial rate (μmoles/min) after *t* min preincubation and v_0 = initial rate without preincubation.

α-Naphthol (μM)	*Santoquin (μM)*	*Decreased activity (%)*	
		Preincubation time:	
		1 min	*8 min*
0		17	65
2.2		7	46
4.4		3	22
8.35		3	18
	2	11	61
	4	1	46
	9.25	1	8

enzyme and not solely related to their traditional antioxidant potencies (some of which are listed in Table I).

We recently reported that eicosa-5,8,11,14-tetraynoic acid inhibited vesicular gland oxygenase in an instantaneous, concentration-dependent manner in addition to causing a time-dependent destruction of the enzyme[11]. When the enzyme was incubated with this acetylenic acid and either α-naphthol or Santoquin, the lowered velocity observed at zero incubation time could be accounted for by the additive effects of the acetylenic acid and the antioxidant. However, the time-dependent destructive action of the tetrayne was prevented (Table III). From these results, it appears that α-naphthol and Santoquin are inhibiting in a different manner than eicosatetraynoic acid which seems to function as a substrate analog.

Our results suggest that a careful evaluation of the effects of dietary antioxidants may show a diminution in prostaglandin synthesis similar to that seen with non-steroidal anti-inflammatory agents[18]. In regard to suggestions that antioxidants may be used to retard the aging process[19,20], we wonder whether this retardation is dependent upon the inhibition of prostaglandin synthesis or occurs in spite of the inhibition.

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