Stimulatory Effect of Histamine on the Peroxidation of Linoleic Acid

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Stimulatory effects of histamine on the peroxidation of linoleic acid vesicle membranes were studied. Among the imidazole-related materials tested, histamine enhanced the peroxidation of linoleic acid best of all. It became apparent that both imidazole and primary amino groups of histamine were required for the stimulatory activity. Histamine significantly enhanced the rate of lipid peroxidation in concentrations less than 10 mM, whereas the stimulatory effect of histamine on the peroxidation of linoleic acid was completely inhibited by the iron chelators diethylenetriamine-*N*,*N*,*N*′,*N*″,*N*″-pentaacetic acid (DTPA) and Desferal. Furthermore, the addition of Fe(II) enhanced significantly the histamine-dependent lipid peroxidation. These results suggest that histamine affects the redox potential of iron, thereby stimulating the iron-dependent lipid peroxidation.

INTRODUCTION

Lipid peroxidation which involves a series of free-radical-mediated chain reaction processes is one of the major causes of food spoilage and has been implicated in numerous pathological and toxicological states (Halliwell and Gutteridge, 1985; Tappel, 1980). In general, lipid peroxidation is an important deteriorative reaction in the processing and storage of foods, causing loss in functional properties and nutritional value. Lipid peroxidation is usually avoided, if possible, but instances do occur where it is encouraged.

A variety of organic and inorganic compounds have been found to stimulate lipid peroxidation (Braughler et al., 1986, 1988; Hicks et al., 1988; Liebler et al., 1986; Minotti and Aust, 1987; Girotti et al., 1985). Amino acids also affect directly the rate of lipid peroxidation. Especially, it has been well characterized that L-histidine significantly affects lipid peroxidation (Tjhio and Karel, 1969; Erickson and Hultin, 1987). We also have investigated the effect of imidazole-related compounds on lipid peroxidation, because of the sensitivity to various oxidizing conditions and the acid–base characteristics of the imidazole side chain at physiological pH and their specific metal binding ability. L-Histidine and its analogues have been found to exhibit significant reactivity with singlet oxygen (Tomita et al., 1969), lipid hydroperoxide (Tjhio and Karel, 1969; Roy and Karel, 1973; Yong and Karel, 1978, 1979), and metal-catalyzed free-radical systems (Uchida and Kawakishi, 1986, 1989, 1990; Uchida et al., 1989). In the course of this study, we have recently found that, among the compounds tested, histamine stimulated most effectively the formation of conjugated dienes from linoleic acid. To our knowledge, the stimulatory effect of histamine on lipid peroxidation has not yet been characterized in detail.

In the present study, we examine the effects on the linoleic acid peroxidation of histamine in detail and present evidence that the stimulatory effect of histamine on the peroxidation of linoleic acid is due to the combined action of iron.

MATERIALS AND METHODS

Materials. *N*-Acetylhistamine, EGTA (ethylene glycol bis-(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid), 1-methylhistamine [1-methyl-4-(β-aminoethyl)imidazole], and carnosine were obtained from Sigma (St. Louis, MO), and L-anserine nitrate was obtained from Calbiochem (La Jolla, CA). Histamine, linoleic acid, and DTPA (diethylenetriamine-*N*,*N*,*N*′,*N*″,*N*″-pentaacetic acid) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Linoleic acid was further purified by vacuum distillation under a nitrogen atmosphere. Desferal was obtained from Ciba-Geigy (Basel, Switzerland). Other chemicals were of analytical grade and were used without further purification.

Peroxidation of Linoleic Acid. Lipid peroxidation was performed in which almost the same experimental conditions of Hicks et al. (1988) were reproduced. In these incubations, the mixtures of linoleic acid (20 mg) and arachidonic acid (0.02 mg) in 4 mL of 0.1 M Tris-HCl buffer, pH 8.0, were thoroughly emulsified with an ultrasonic vibrator for 1 min and then diluted to 20 mL with the same buffer. The mixtures of fatty acids were always freshly prepared. The mixtures containing linoleic acid (1 mg), arachidonic acid (1 μg), and histamine or its analogues (1 mM) in 1.5 mL total volume were allowed to stand at 40 °C in the dark.

Assay of Linoleic Acid Peroxidation. Unless otherwise noted, peroxidation of linoleic acid was assessed by an increase in 234-nm absorbance due to conjugated diene formation by using a molar extinction coefficient of 2.8×10^4 M^{-1} cm^{-1}. The histamine-dependent consumption of linoleic acid was determined by HPLC reverse-phase chromatography. Reaction mixtures were clarified by the addition of an equal volume of methanol, and the resulting solution was applied to a Develosil ODS-5 column (0.46 × 25 cm). Linoleic acid was eluted with a solution of 95% methanol in 0.1% trifluoroacetic acid at a rate of 0.8 mL/min, the elutions being monitored by absorbance at 210 nm.

RESULTS AND DISCUSSION

The effect of various imidazole-related materials on the peroxidation of linoleic acid was examined (Table I). The result shows that the compounds tested at 1 mM were found to differ markedly in their activities. Obviously, histamine has an outstanding prooxidant effect on lipid peroxidation. We have also confirmed that histamine showed stimulatory effect in another lipid peroxidation system, the iron(II)/ADP/ascorbate-dependent peroxidation of arachidonic acid (Uchida et al., unpublished result). Only L-histidine was able to stimulate the reaction at a rate comparable to that of histamine. In relation to this, Tjhio and Karel (1969) and Erickson and Hultin (1987) have demonstrated the prooxidant effect of histidine and have characterized the effect of various transition-metal ions on the histidine-dependent lipid peroxidation. Other imidazole-related materials that showed a distinct positive action were imidazole, 1-methylimidazole, and 1-acetylimidazole; however, their responses were

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Table I. Effect of Imidazole-Related Materials on Linoleic Acid Peroxidation

addition[a]	dienes,[b] nmol/mL	% of control
none (control)	621 ± 73	100
α-tocopherol	218 ± 8	35
imidazole	780 ± 19	125
1-methylimidazole	780 ± 6	125
2-methylimidazole	361 ± 16	58
4-methylimidazole	465 ± 41	75
1-acetylimidazole	785 ± 8	126
5,6-dimethylbenzimidazole	315 ± 10	51
histamine	1352 ± 2	217
N-acetylhistamine	646 ± 16	104
1-methylhistamine	787 ± 28	126
L-histidine	1098 ± 1	177
anserine	492 ± 11	79
carnosine	274 ± 15	44

[a] Concentration of the additions is 1 mM. [b] Conjugated dienes from linoleic acid were measured after 24 h of incubation, and values are indicated as means ± SD of duplicate incubations from two separate experiments.

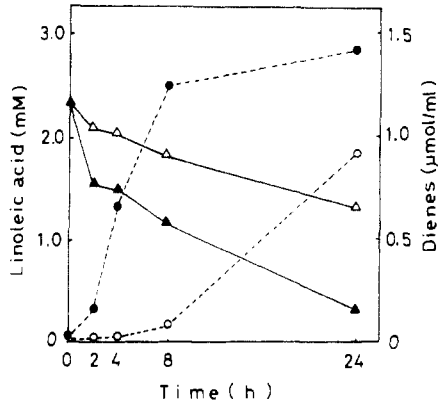

Figure 1. Stimulation of the peroxidation of linoleic acid by histamine. Consumption of linoleic acid was monitored by HPLC reverse-phase chromatography. Formation of conjugated dienes was measured by UV absorbance at 234 nm. Symbols: ●, ○ conjugated diene formation; ▲, △ residual linoleic acid; open symbols indicate no histamine.

much slower than that of histamine. Both *N*-acetylhistamine and 1-methylhistamine failed to show any activity, suggesting that both imidazole and primary amino groups of histamine are essential to the occurrence of activity. In contrast, it is of interest to note that carnosine (β-alanyl-L-histidine) exhibited a potent antioxidant activity in linoleic acid peroxidation, which was comparable to that of the well-known antioxidant α-tocopherol. 2-Methylimidazole, 4-methylimidazole, 5,6-dimethylbenzimidazole, and anserine (β-alanyl-L-1-methylhistidine) were also slightly antioxidative.

As shown in Figure 1, loss of linoleic acid and concomitant formation of conjugated dienes were significantly enhanced by the addition of histamine. In the absence of histamine, the rate of peroxidation of linoleic acid was rather slow, and only 35% of the substrate was consumed after 24 h of incubation, while the addition of 1 mM histamine led to a rapid stimulation of lipid peroxidation. Thus, at the end of 24 h, more than 80% of the substrate was consumed.

The effect of varying concentrations of histamine on stimulation of linoleic acid peroxidation is shown in Figure 2. A marked increase in the concentration of conjugated dienes was observed when the histamine concentration was raised to 1–5 mM histamine, whereas relatively high concentrations of histamine, above 10 mM, failed to enhance the peroxidation.

These results seem to suggest that, without any catalysts,

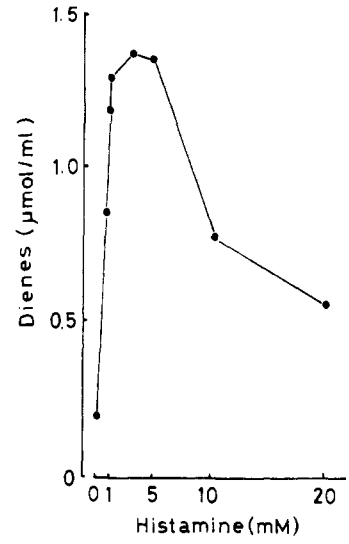

Figure 2. Effect of histamine concentrations on the peroxidation of linoleic acid. Conjugated dienes from linoleic acid were measured after 24 h of incubation.

Table II. Effect of Metal Chelating Agents on the Histamine-Dependent Peroxidation of Linoleic Acid

addition[a]	dienes,[b] nmol/mL	% of control
histamine (control)	1201 ± 5	100
histamine + ADP	1216 ± 56	101
histamine + ATP	1109 ± 77	92
histamine + EDTA	567 ± 25	47
histamine + EGTA	897 ± 5	75
histamine + DTPA	223 ± 19	19
histamine + Desferal	207 ± 21	17

[a] Concentrations of histamine and the chelators are 1 mM and 10 μM. [b] Conjugated dienes from linoleic acid were measured after 24 h of incubation, and values are indicated as means ± SD of duplicate incubations from two separate experiments.

histamine stimulates lipid peroxidation by enhancing the formation of lipid hydroperoxide during the process. However, it is unlikely that histamine alone can initiate or promote lipid peroxidation. We supposed that an ambiguous catalyst was involved in the reaction, and the most probable catalyst was assumed to be metal ion. Hence, we determined the role of metal ion in the histamine-dependent lipid peroxidation. The inhibitory effects of metal chelators on the reaction are presented in Table II. The result demonstrates that the iron chelators DTPA and Desferal largely inhibited the peroxidation, whereas the other chelators were less effective. Both DTPA and Desferal might bind tightly with iron and might reduce the redox potential of the metal in lipid peroxidation. In relation to this, it has been demonstrated that complexes of iron with DTPA are reduced only slightly by reductant and show diminished reactivity in iron-dependent $^{\bullet}OH$ production (Halliwell, 1978). In addition, Desferal forms Fe(III) complex tightly, which is also a powerful inhibitor of iron-dependent lipid peroxidation (Wills, 1969). Other than iron ion, we have determined the effect of addition of metal ions such as Cu(II), Mn(II), Ti(III), and Co(II) on the histamine-dependent lipid peroxidation: they were all inactive (data not shown).

Then we examined the effect of Fe(II) addition on the histamine-dependent lipid peroxidation (Table III). The result clearly demonstrates that the histamine-dependent lipid peroxidation is further enhanced by the addition of Fe(II) or Fe(II)/EDTA. Consequently, it is evident that the stimulatory effect of histamine on lipid peroxidation is due to the combined action of iron.

These results clearly demonstrate an involvement of iron

Table III. Effect of Iron on the Histamine-Dependent Peroxidation of Linoleic Acid

addition[a]	dienes,[b] nmol/mL	% of control
none	56 ± 7	100
Fe(II)	72 ± 5	129
Fe(II)/EDTA	117 ± 6	209
histamine	345 ± 27	616
histamine/Fe(II)	729 ± 41	1302
histamine/Fe(II)/EDTA	733 ± 17	1309

[a] Concentrations of the additions are 10 μM Fe(II), 10 μM EDTA, and 1 mM histamine. [b] Conjugated dienes from linoleic acid were measured after 2 h of incubation, and values are indicated as means ± SD of duplicate incubations from two separate experiments.

in the histamine-mediated lipid peroxidation. A detailed mechanism of the reaction has not been characterized yet; however, at present, we assume that the formation of an equivocal iron–histamine complex is the most probable explanation for the histamine-mediated lipid peroxidation. In relation to this, certain iron chelates are known to accelerate the autoxidation of Fe(II) and the generation of free radicals (Halliwell, 1978), which can abstract the hydrogen atom from lipid, resulting in the formation of lipid peroxides.

The effect of histidine, histamine, and other imidazole-related compounds on lipid peroxidation has been examined previously (Tjhio and Karel, 1969; Kohen et al., 1988; Erickson and Hultin, 1987). It has been shown that most of the imidazole-related compounds including histamine work as antioxidant (Kohen et al., 1988; Babizhayev, 1989). Erickson and Hultin (1987) have shown that the activity of histidine could be prooxidant or antioxidant, and they have found that this is dependent on the order of addition of the compounds. Hence, we have determined the order of addition of histamine and iron; however, we could not find any significant difference at all (data not shown). We therefore consider that, at least in our model system, the rate of lipid peroxidation mediated by histamine is highly dose dependent (Figure 2). It is thus tempting to speculate that histamine has some influence on iron and creates a more favorable Fe(II)/Fe(III) redox cycle and/or facilitates the initiation step of lipid peroxidation.

This study has been mainly focused on the prooxidant activity of histamine, while some of the histidine-related materials, such as carnosine and anserine, exhibited antioxidant activity (Table I). In regard to this, Kohen et al. (1988) have demonstrated that carnosine and its analogues possess several of these antioxidant properties in chemically induced lipid peroxidation. According to their study, carnosine and its analogues can act as the scavengers of free radicals and efficient chelating agents for transition metals.

Thus, the imidazole-related materials have various attractive functions in lipid peroxidation. We believe that these results of model experiments can be directly extrapolated to food systems, since not only linoleic acid but histamine by itself are normal food components; in addition, free fatty acid micelles are also normally formed in various food systems. Our results therefore raise the possibility that lipid peroxidation mediated by histamine may occur during food preservation or processing. Further study will be needed to define more clearly the mechanism of the histamine-dependent lipid peroxidation in food systems.

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