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RELATIONSHIP BETWEEN LIPID PEROXIDATION AND PROSTAGLANDIN GENERATION IN RABBIT TISSUES

JADWIGA ROBAK and BARBARA SOBAŃSKA
Department of Pharmacology, Copernicus Medical Academy, Cracow 31–531, Grzegórzecka 16, Poland

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Abstract—Lipid peroxides are formed non-specifically from unsaturated lipids and specifically in the course of prostaglandin biosynthesis. Both kinds of peroxidation were found to be interrelated in homogenates of the renal medulla, lungs and spleen of rabbits but not in homogenates of the renal cortex and brain. Lipid peroxidation was increased by ascorbic acid and ferrous ions, especially in the renal cortex, brain and renal medulla homogenates. Arachidonic, but not oleic, acids increased lipid peroxidation in the renal medulla, but this augmentation is inhibited by indomethacin. Arachidonic, as well as oleic, myristic and linolenic acids, markedly depressed lipid peroxidation in the renal cortex. It is concluded that lipid peroxidation in the renal medulla is mainly specific and in the renal cortex mainly non-specific.

Non-specific peroxidation of a number of unsaturated lipids is promoted by ascorbic acid, ferrous ions and NADPH [1]. A more specific formation of peroxides limited to the 20-C unsaturated fatty acids takes place during the enzymic generation of prostaglandins [2]. All these lipid peroxides can be broken down to malondialdehyde [2,3], the estimation of which has been proposed either for quantification of non-specific lipid peroxidation [4] or for the evaluation of prostaglandin synthetase activity [5,6]. We wondered whether the intensity of the lipid peroxidation (as measured by malondialdehyde formation) is related to the prostaglandin synthetase activity in various tissues.

MATERIALS AND METHODS

Animals. Rabbits of both sexes, weighing 2000–3000 g were killed by a blow on the neck. The kidneys, brain, spleen and lungs were quickly removed and placed in a dry container in an ice-bath.

Reagents. Malondialdehydetetraethyl acetal, from K & K Lab. Inc. Plainview, USA. Arachidonic acid, Sigma Chemical Co. USA. Myristic acid, Koch–Light Laboratories, England. Oleic acid, Argon, Poland. Linolenic acid, Fluka A. G. Buchs, Switzerland. Other reagents were obtained from commercial sources. Fatty acids and indomethacin we added to the solutions in form of sodium salts.

Ascorbic acid content in tissues. The method of Roe and Kuether [7] was used.

Lipid content in tissues. Lipids were extracted with a mixture of chloroform and methanol [8], separated and weighed. The lipid content in tissues was expressed in mg of lipid extract per g of wet tissue.

Lipid peroxidation. The procedure of Utley [9] was used. Tissues were homogenized at 4° in 0.067 M phosphate buffer, pH 7.4, and made up to a concentration of 30 mg/ml. These homogenates were incubated by shaking at 37° for 90 min in 2-ml samples. Proteins were precipitated with 1 ml 20% trichloracetic acid and centrifuged at 20,000 *g* for 10 min. The supernatant was treated with 1 ml 0.067% sodium thiobarbiturate solution and heated in boiling water for 10 min. The developed colour was read on a Spekol colorimeter at 530 nm against a sample treated with trichloracetic acid before incubation. The malondialdehyde formed was quantified from the standard graph drawn for malondialdehyde–tetraethyl acetal and calculated either for 1 g wet tissue or for 1 g tissue lipids. The molar extinction coefficient was 1.35×10^5.

Lipid peroxidation in kidney microsomes. The kidney medulla and cortex were separated and homogenized in 0.067 M phosphate buffer at pH 8 (30% w/v). The homogenates were centrifuged at 20,000 *g* for 10 min and the supernatant was centrifuged once more at 105,000 *g* for 60 min. The 105,000 *g* pellets were suspended in distilled water and the protein content in the suspension was determined by the biuret method [10]. Lipid peroxidation was estimated after 10 min incubation of the mixture containing kidney medulla or cortex microsomes (5 mg microsomal protein/ml), ferrous sulphate (100 μM), ascorbic acid (1000 μM) in 0.067 M phosphate buffer at pH 7. Control samples contained neither ferrous sulphate nor ascorbic acid.

Prostaglandin-synthesizing potency of homogenates. A 1000 *g* Supernatant of tissue homogenates (6.7% w/v) in 0.067 M phosphate buffer at pH 8 was incubated for 5 min at 37° in 2-ml samples. The enzymic reaction was stopped by boiling and the samples were centrifuged at 10,000 *g* for 15 min. Prostaglandin-like activity was bioassayed in the supernatant according to the method of Vane [11] and expressed as PGE_2-equivalents (ng) per g of wet tissue.

Statistical and analysis. Student's *t*-test was used and S.E. is shown in the figures.

RESULTS

The intensity of lipid peroxidation in tissue homogenates might be related to their endogenous levels of lipids or ascorbic acid. No such direct interrelationship, however, was found in our experiments for

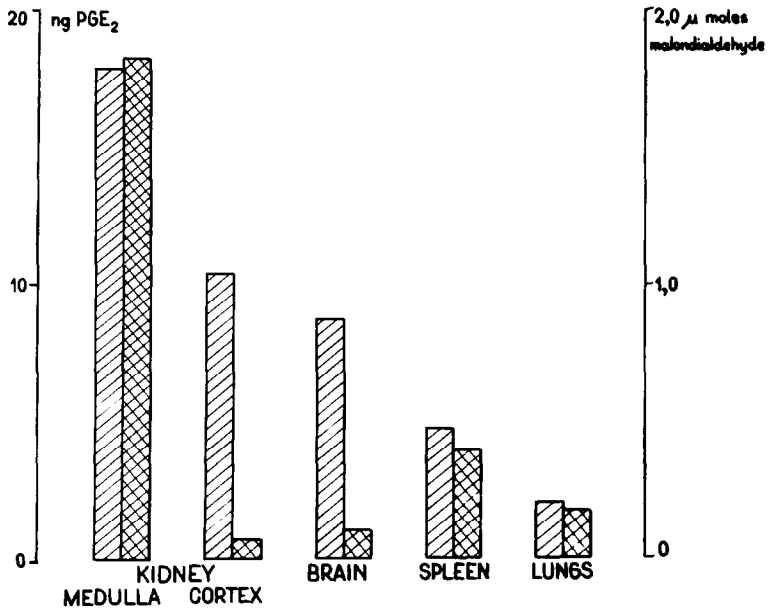

Fig. 1. Lipid peroxide formation and prostaglandin synthesizing potency in homogenates of rabbit tissues. Hatched columns represent the amount of malondialdehyde formed during incubation of homogenates, calculated in μmoles per g lipids. Cross-hatched columns represent ng prostaglandin like activity expressed as PGE_2 per g tissue.

Table 1. Lipid peroxidation (i.e. malondialdehyde formation), lipids and ascorbic acid contents in rabbit tissue homogenates

Tissue	Malondialdehyde (μmoles/g tissue)	Lipids (mg/g tissue)	Ascorbic acid (μmoles/g tissue)
Kidney cortex	0.031 ± 0.019 n = 14	29.7 ± 3.7 n = 3	0.23 ± 0.066 n = 3
Kidney medulla	0.024 ± 0.003 n = 16	13.3 ± 3.7 n = 3	0.38 ± 0.077 n = 3
Brain	0.09 ± 0.018 n = 7	101.7 ± 4.4 n = 3	1.26 ± 0.28 n = 3
Spleen	0.013 ± 0.002 n = 4	27.3 ± 3.7 n = 3	1.94 ± 0.18 n = 3
Lungs	0.007 ± 0.003 n = 5	36.7 ± 4.5 n = 3	1.00 ± 0.034 n = 3

lipid peroxidation and ascorbic acid content ($r = 0.0345$). On the other hand, there is a correlation between the lipid peroxidation and lipid content in the tissue ($r = 0.8960$) (Table 1). We therefore decided to present the intensity of lipid peroxidation in terms of malondialdehyde formed by 1 g of tissue lipids. The intensities of lipid peroxidation and prostaglandin generating potencies in various tissues are compared in Fig. 1. Of all the tissues, kidney medulla had the largest lipid peroxidation and prostaglandin synthesizing capacities. Renal cortex and brain homogenates produced small amounts of prostaglandins in spite of large amount of malondialdehyde. The correlation coefficient between lipid peroxidation and prostaglandin synthetizing potency was 0.777 for all five tissues tested.

The influence of ascorbic acid, ferrous ions and arachidonic acid on lipid peroxidation in rabbit tissues is shown in Fig. 2. Ascorbic acid alone (850 μM) has little (renal medulla) or no (renal cortex) influence on lipid peroxidation in the kidney, but ascorbic acid

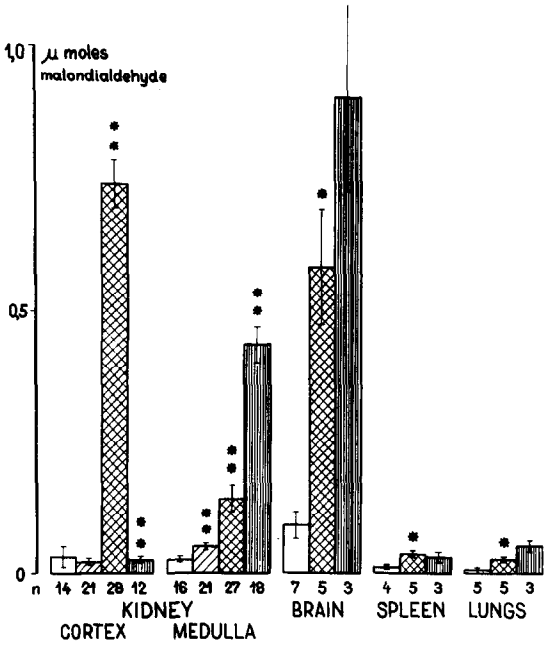

Fig. 2. The influence of ascorbic acid (hatched columns), ferrous ions with ascorbic acid (cross-hatched columns) and both these reagents with arachidonic acid (stripped columns) on lipid peroxidation in rabbit tissues. The concentrations of reagents were as follows: ascorbic acid 850 μM, ferrous sulphate 30 μM, arachidonic acid 2000 μM. The results are expressed in μmoles of malondialdehyde formed by 1 g tissue during 90 min. The results, expressed as hatched and cross-hatched columns, were compared with control (white columns) and the results with arachidonic acid (striped columns) with the same samples without arachidonic acid (cross-hatched columns). ** Denotes $P < 0.001$; * Denotes $0.01 > P > 0.001$.

Table 2. The influence of fatty acids in concentrations of 2 mM on lipid peroxide formation in rabbit kidney homogenates containing 30 μM ferrous sulphate and 850 μM of ascorbic acid

Fatty acid	Kidney: Cortex	n	Kidney: Medulla	n
None	0.745 ± 0.045	28	0.144 ± 0.024	27
Arachidonic	* 0.026 ± 0.005	12	* 0.436 ± 0.052	18
Oleic	* 0.052 ± 0.004	3	0.113 ± 0.028	3
Myristic	* 0.023 ± 0.007	3	0.132 ± 0.015	5
Linolenic	* 0.005 ± 0.005	3	* 0.055 ± 0.007	6

The results are expressed in μM of malondialdehyde formed by 1 g tissue during 90 min.

* Denotes statistical significance, $P < 0.001$.

in conjuction with exogenous ferrous ions (30 μM) results in an increase of lipid peroxidation in all the tissues tested in the following order of intensity: renal cortex, brain, renal medulla, lungs and spleen. Figure 2 also shows that malondialdehyde formation induced by ascorbic acid is depressed by arachidonic acid in a concentration of 2 mM in the renal cortex, increased in the renal medulla, and unchanged in the brain, spleen and lungs.

To check the specificity of the arachidonic acid effects on lipid peroxidation in both zones of the kidney we have also tested oleic, myristic and linolenic acids. These acids did not stimulate malondialdehyde formation in the renal medulla but were still powerful depressants of lipid peroxidation in the kidney cortex (Table 2). The effects of arachidonic and oleic acids on lipid peroxidation in both zones of the kidney are dose-dependent (Fig. 3).

Figure 4 shows the influence of indomethacin at a concentration of 1 mM on lipid peroxidation in the renal cortex and medulla. In the absence of exogenous arachidonic acid this prostaglandin synthetase inhibitor did not decrease malondialdehyde formation, but in the presence of 2 mM of arachidonic acid indomethacin increased lipid peroxidation in the kidney cortex and decreased it in the renal medulla.

When microsomal preparations of kidney were used, arachidonic acid at a concentration of 2 mM had no influence on lipid peroxidation in the renal cortex (from 56 ± 6 to 67 ± 6 nmoles of malondialdehyde per mg of microsomal protein) but it stimulated lipid peroxidation in the renal medulla microsomes (from 87 ± 2 to 206 ± 36 nmoles of malondialdehyde per mg of microsomal protein).

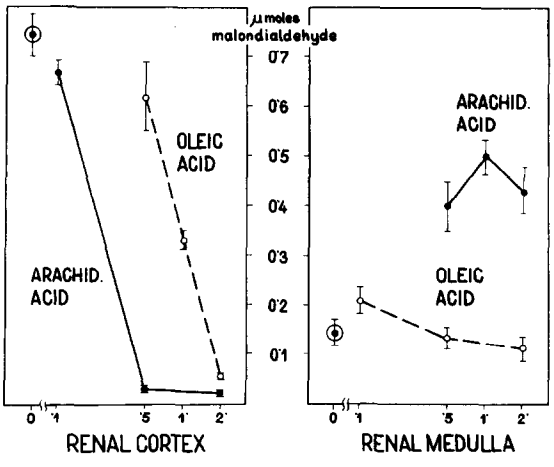

Fig. 3. The influence of arachidonic acid (dark circles) and oleic acid (light circles) on lipid peroxidation in homogenates of the renal cortex and medulla. The incubation mixture contained tissue homogenates with 30 μM of ferrous sulphate, 850 μM of ascorbic acid and a solution of sodium salts of fatty acids. Ordinate: fatty acid concentration in mM. Abscissa: lipid peroxidation expressed as μmoles of malondialdehyde formed in an amount of homogenate equivalent to 1 g tissue during 90 min.

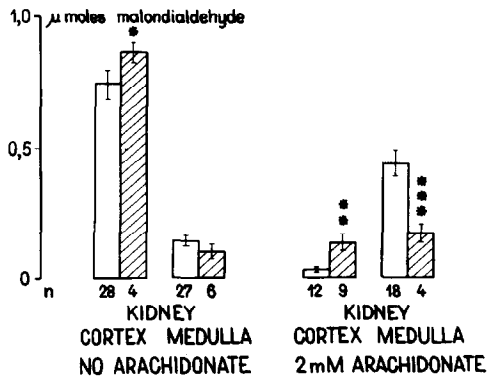

Fig. 4. The influence of indomethacin (hatched columns) on native (two groups of bars on left) and arachidonic acid—induced (two groups of bars on right) lipid peroxidation in homogenates of rabbit kidneys in the presence of 850 μM of ascorbic acid and 30 μM of ferrous sulphate. Homogenates were preincubated at room temperature without (white columns) or with (hatched columns) 1 mM of indomethacin. They were then incubated with or without arachidonic acid for 90 min at 37°. Lipid peroxidation is expressed in μmoles of malondialdehyde formed by 1 g tissue. The results were compared with those without indomethacin and showed: * $0.05 > P > 0.01$; ** $0.01 > P > 0.001$; *** $0.001 > P$.

DISCUSSION

The intensities of malondialdehyde and prostaglandin generation in the renal cortex and in the brain homogenates do not seem to be related to each other, contrary to the evident correlation of lipid peroxidation and prostaglandin formation in the renal medulla, lungs and spleen. The renal cortex and brain have high lipid peroxidation potenties and at the same time the lowest prostaglandin synthetizing potency per g wet tissue. Therefore it seems that most of the lipid peroxides formed in these tissues are not related to the oxygenation of the 20-C unsaturated fatty acids, which are the substrates for prostaglandin synthetase and the formation of endoperoxides [2,12–15]. Small amounts of prostaglandins found in kidney cortex are a result of high prostaglandin dehydrogenase activity in this tissue. Low prostaglandin-synthesizing potency in brain homogenates (expressed as PGE-like activity) may be explained by prostaglandin $F_{2\alpha}$ (Schaeffer and Seregi, personal

communication) or thromboxane [16] formation in this homogenate.

The most potent prostaglandin synthesizing capacity was found in the renal medulla. This high activity may explain the fact that only in this tissue did arachidonic acid stimulate lipid peroxidation both in homogenates and in the microsomal preparation. This stimulation is inhibited by indomethacin, a prostaglandin synthetase inhibitor [17–19]. A similar finding was reported in the microsomal preparation of bovine seminal vesicle microsomes [5,6]. This inhibition was not observed in microsomes of rat brain (Schaeffer and Seregi, personal communication) or in homogenates of rabbit kidney cortex (our present experiments). The seminal vesicles and renal medulla are the richest sources of prostaglandin synthetase in the body [20–23]. It is highly possible, therefore, that in these tissues malondialdehyde comes mainly from endoperoxide formed by prostaglandin synthetase and thus its formation is promoted by arachidonic acid and inhibited by indomethacin. In prostaglandin synthetase-poor tissues malondialdehyde is produced mostly from unspecific peroxides, and prostaglandin synthetase inhibitor had no effect upon it.

In renal cortex homogenates, but not in microsomes, all the fatty acids tested, including arachidonic acid, markedly depress lipid peroxidation. The mechanism of this effect is not clear. It may be that the detergent properties of sodium salts of fatty acids are responsible. Wills [1] found inhibition of lipid peroxidation by some detergents. It may also be that the excess of substrate (lipids) inhibits unspecific lipid peroxidation.

Concluding remarks

1. There exists a relation between the potencies of lipid peroxidation and prostaglandin-synthesizing capacity in homogenates of rabbit renal medulla, lungs and spleen, but not in homogenates of the renal cortex and brain.

2. It is concluded that in tissues rich in prostaglandin synthetase malondialdehyde is formed mainly from cyclic endoperoxides, whereas in tissues poor in prostaglandin synthetase lipid peroxides detected as malondialdehyde originate from linear hydroperoxides of fatty acids.

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