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DECREASED LIPID PEROXIDATION IN THE RAT KIDNEY DURING GESTATION

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Renal malonaldehyde content and lipid peroxidation, induced by ascorbate, NADPH and cumene hydroperoxide, are significantly decreased during gestation in rats. Lipid peroxidation tends to reach normal levels in the kidney post partum. In the renal mitochondria lipid peroxidation without co-factors and that induced by cumene hydroperoxide, ascorbate and NADPH is decreased during pregnancy. However, in the microsomes, only lipid peroxidation induced by NADPH and cumene hydroperoxide is affected. The observed decrease in lipid peroxidation during gestation is reflected by low levels of total lipid and phospholipid.Endogenous inhibitors of lipid peroxidation also increase during pregnancy. © 1987 Academic Press, Inc.

Gestation in mammals encompasses a variety of complex biochemical reactions. Growth of maternal organs during gestation is well documented (1). Lipid peroxidation is a basic reaction involved in membrane alterations during normal physiological processes (2). Rapid growth of organs due to cell proliferation during pre and postnatal development (3-5) and regeneration (6) are characterized by low levels of lipid peroxidation. Uncontrolled growth during malignant transformation is also associated with inhibition of lipid peroxidation (7). Unlike these, growth of maternal organs seems to be due to hypertrophy of cells rather than hyperplasia (1).

Though there are several reports on the growth of maternal organs and accompanying biochemical changes during pregnancy (1), there is a paucity of data on the level of lipid peroxidation in maternal organs during gestation. In the present study, we have examined lipid peroxidation in kidney and the factors influencing it during pregnancy.

MATERIALS AND METHODS

Preparation of tissue fractions

3-Month-old Wistar rats were used for these studies. Virgin females were mated with males and the day on which sperms were detected in the vaginal smears was designated Day 1 of pregnancy. Rats were killed by cervical dislocation and kidneys were homogenized in 0.15 M Tris-HCl buffer, pH 7.4. Mitochondria and microsomes, prepared as described earlier (8), were suspended in the above buffer at a concentration of 5 mg protein/ml for further analyses.

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Lipid peroxidation systems

The modified thiobarbituric acid (TBA) method (9) was used for the estimation of malonaldehyde content in the freshly prepared tissue fractions. The assay (0.5 ml) for ascorbate-induced lipid peroxidation, as described earlier (10), contained 50 µl tissue sample, 50 µM $FeSO_4$, 1 mM KH_2PO_4 and 0.4 mM ascorbic acid in 0.15 M Tris-HCl buffer, pH 7.4. NADPH-induced system contained 50 µl tissue sample, 50 µM $FeCl_3$, 5 mM ADP, 1 mM KH_2PO_4 and 0.4 mM NADPH in the above buffer. For cumene hydroperoxide-induced lipid peroxidation, the assay (0.5 ml) contained 50 µl tissue sample, 0.5 mM cumene hydroperoxide, 0.05 M Tris-HCl, pH 7.4. Lipid peroxidation without co-factors was performed by incubating the tissue fractions in 0.15 M Tris-HCl buffer, pH 7.4. The assay mixtures were incubated at 37°C in a shaker water bath and the malonaldehyde formed was estimated by the modified TBA method (10).

Factors related to lipid peroxidation

For the estimations of total lipid, phospholipid, cholesterol, ascorbic acid, α-tocopherol and reduced glutathione, standard methods, as cited earlier (10,11), were used.

RESULTS

A significant increase in the wet weight of kidney is observed during gestation (Table 1). But the total protein content is not altered. Lipid peroxidation assayed with ascorbate-, NADPH- and cumene hydroperoxide is decreased during gestation. Malonaldehyde content also shows a similar trend. Ascorbate- and cumene hydroperoxide-induced lipid peroxidation and the malonaldehyde content are low during the first half of pregnancy (Fig.1). These values tend to reach normal levels 3 days post partum. NADPH-induced

Table 1

Tissue weight and lipid peroxidation in kidneys of non-pregnant and pregnant rats

Parameter	Non-pregnant	Pregnant
Kidney weight[1]	1.28 ± 0.06	1.68 ± 0.12[A]
Protein content[2]	106.68 ± 11.23	105.28 ± 8.01
Malonaldehyde content[3]	1.53 ± 0.32	0.68 ± 0.05[A]
Lipid peroxidation		
without cofactors[4]	2.23 ± 0.11	2.15 ± 0.41
with ascorbate-induced system[4]	20.94 ± 1.39	13.44 ± 0.75[B]
with NADPH-induced system[4]	9.15 ± 0.71	5.30 ± 0.26[B]
with cumene hydroperoxide[4]	3.80 ± 0.33	1.84 ± 0.29[B]

Values are means ± S.E. from 5 rats and are expressed as [1] g/animal, [2] mg/g kidney, [3] n mol malonaldehyde/mg protein and [4] n mol malonaldehyde/mg protein after incubation at 37°C in a shaker water bath for 30 min. [A] $P < 0.01$ and [B] $P < 0.001$, as compared with non-pregnant rats.

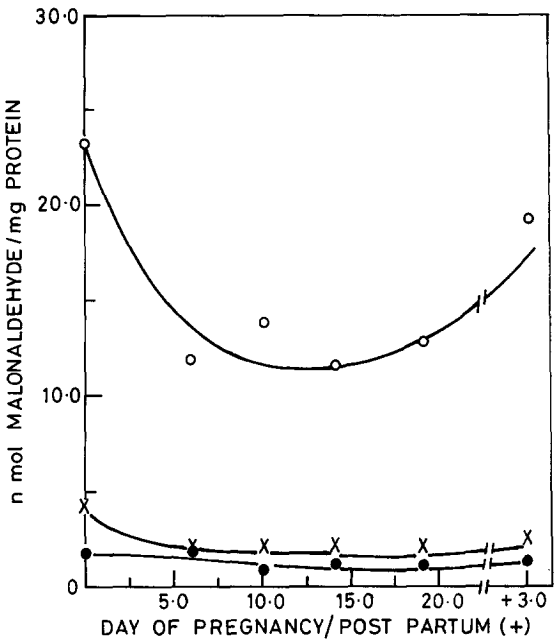

Fig.1. Lipid peroxidation in rat renal homogenate as a function of gestational period. Values are means from 5 rats and represent malonaldehyde (n mol/mg protein) content (●), lipid peroxidation (n mol malonaldehyde/mg protein/30 min) with ascorbate-induced system (○) and cumene hydroperoxide (x). The incubations were carried out as described in 'Materials and methods'

lipid peroxidation, however, does not show any particular pattern as a function of gestation time (data not included).

Data on the lipid peroxidation in renal mitochondria and microsomes of non-pregnant and pregnant rats are presented in Table 2. Malonaldehyde content and

Table 2

Lipid peroxidation in renal mitochondria and microsomes of pregnant and non-pregnant rats

Parameter	Mitochondria		Microsomes	
	Non-pregnant	Pregnant	Non-pregnant	Pregnant
Malonaldehyde content[1]	1.89 ± 0.18	1.00 ± 0.16[B]	2.89 ± 0.2	2.89 ± 0.47
Lipid peroxidation				
without cofactors[2]	3.57 ± 0.71	1.47 ± 0.21[A]	7.83 ± 0.68	8.62 ± 1.36
with ascorbate-induced system[2]	45.70 ± 7.71	23.52 ± 0.79[A]	41.52 ± 2.06	45.47 ± 6.28
with NADPH-induced system[2]	9.90 ± 1.52	4.41 ± 1.21[A]	7.26 ± 0.33	4.65 ± 0.43[B]
with cumene hydroperoxide[2]	4.86 ± 1.40	1.37 ± 0.25[A]	17.24 ± 1.02	9.64 ± 1.59[B]

Values are means ± S.E. from 5 rats and are expressed as [1] n mol malonaldehyde/mg protein in the freshly prepared renal subcellular fractions and [2] n mol malonaldehyde/mg protein after incubation at 37°C in a shaker water bath for 30 min. [A] $P < 0.05$ and [B] $P < 0.01$ as compared with non-pregnant rats.

Table 3
Factors related to lipid peroxidation in kidneys of pregnant and non-pregnant rats

Factor	Non-pregnant	Pregnant
Total lipid[1]	481 ± 56	320 ± 34[A]
Phospholipid[1]	353 ± 8	253 ± 17[B]
Ascorbic acid[1]	1.32 ± 0.23	1.97 ± 0.18
Reduced glutathione[1]	6.79 ± 0.52	9.38 ± 0.78[A]
α-Tocopherol[1]	0.65 ± 0.19	2.00 ± 0.36[A]
Cholesterol : Phospholipid ratio	0.139	0.079

Values are means ± S.E. from 5 rats and are expressed as [1] μg/mg protein.
[A] $P < 0.05$ and [B] $P < 0.001$ as compared to non-pregnant rats.

lipid peroxidation with ascorbate, NADPH and cumene hydroperoxide and that without co-factors are low in renal mitochondria of pregnant rats. In the microsomes, however, lipid peroxidation induced by NADPH and cumene hydroperoxide is affected.

Table 3 presents data on the factors related to lipid peroxidation in the renal homogenate of non-pregnant and pregnant rats. Total lipid and phospholipid content are lower in the kidneys of pregnant rats. Levels of α-tocopherol and reduced glutathione are significantly higher in the kidneys of pregnant rats. Alteration in the level of ascorbic acid in pregnant rats, however, is not significant. Cholesterol : phospholipid ratio decreases in pregnant rats.

DISCUSSION

Low levels of lipid peroxidation are observed during rapid growth (3-6,12,13). Gestation is associated with overall growth of several maternal organs (1). Enlargement of liver during pregnancy has been shown to be due to hypertrophy rather than hyperplasia (1). The observed growth in kidney is concomitant with a decrease in malonaldehyde content, a measure on in vivo lipid peroxidation (16). The lipid peroxidation potential studied by employing three different systems also decreases. In spite of the differences in mechanism involved (17-19), lipid peroxidation in the kidney, induced by the three systems, is significantly decreased during gestation.

Lipid peroxidation in renal mitochondria shows a different response than that of microsomes during gestation. The difference in their membrane composition may be a factor responsible for this (20). Alterations in the renal lipid peroxidation observed during gestation seem to be abolished after parturition. This indicates the possible influence of hormones elaborated during pregnancy on the control mechanisms involved.

Phospholipids are the major substrates for lipid peroxidation in membranes. Antioxidants such as α-tocopherol and reduced glutathione inhibit lipid peroxidation (20,21). Ascorbic acid, depending on its relative concentration to α-tocopherol, may act as an antioxidant or prooxidant resulting in inhibition or enhancement of lipid peroxidation (22). Our studies show that a decrease in the content of total lipids and phospholipids during gestation is accompanied by a significant increase in the content of α-tocopherol and reduced glutathione.

During gestation a variety of complex biochemical reactions occur in the body. Lipid peroxidation has been suggested as a key factor involved in the breakdown and turnover of biological membranes (18,19,21). Low potential for lipid peroxidation, besides being a possible factor contributing to cell growth, may also be an adaptation so as to reduce the chances of unfavourable alterations in the biological membranes involved in these biochemical reactions.

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