Low level of lipid peroxidation in newborn rats

Possible factors for resistance in hepatic microsomes

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Hepatic rough and smooth microsomes of newborn rats show less sensitivity to ascorbate- and NADPH-induced lipid peroxidation as compared to those of adult rats. Though optimum concentrations of Fe^{2+}, ascorbate and Fe^{3+} significantly increase lipid peroxidation in both age groups, the lipid peroxidation observed in newborns is much less compared with that of adults. Microsomal fractions from newborn rats contain significantly lower amounts of phospholipid, NADPH cytochrome *c* reductase, cytochrome P-450 and a lower degree of unsaturation in lipids. These fractions also exhibit high cholesterol:phospholipid ratios. The resistance to lipid peroxidation observed in the newborns appears to be due to the low availability of substrate and high cholesterol:phospholipid ratio.

(Newborn rat) *Microsomal fraction* *Ascorbate* *NADPH* *Lipid peroxidation* *Phospholipid*

1. INTRODUCTION

Lipid peroxidation has been considered as a basic reaction involved in membrane damage [1,2]. The in vivo lipid peroxidation as measured by expired gases seems to be higher in the case of newborn infants and rabbits as compared to the adults [3]. Similar high levels of lipid peroxidation have also been observed in rat liver homogenate [4] and human blood [5] during the neonatal period. These observations have been attributed to the sudden exposure of animals immediately after parturition to oxygen and oxygen free radicals and the lack of proper defences to protect the membranes from lipid peroxidation [6]. However, there is a paucity of detailed reports pertaining to lipid peroxidation in specific subcellular organelles of newborn animals. This study relates to lipid peroxidation in purified hepatic rough and smooth microsomes of newborn rats compared with that of adults.

2. MATERIALS AND METHODS

2.1. *Animals and preparation of microsomal fractions*

Newborn rats (less than 24 h old) and adult rats (11 weeks old) of the Wistar strain were used. Rough and smooth microsomes were prepared as in [7]. For preparation of microsomal fractions, livers from 25 newborn rats were pooled to form one batch. The rough and smooth microsomes were purified as reported [7] and resuspended in 0.15 M Tris-HCl buffer, pH 7.4, at 5 mg protein/ml.

2.2. *Lipid peroxidation*

The assay system for NADPH-induced lipid peroxidation (0.5 ml) contained 50 μl microsomes, 50 μM $FeCl_3$, 4 mM ADP, 1 mM KH_2PO_4, and 0.4 mM NADPH in 0.15 M Tris-HCl buffer, pH 7.4. For ascorbate-induced lipid peroxidation, the incubation medium (0.5 ml) contained 50 μl microsomes, 50 μM $FeSO_4$, 1 mM KH_2PO_4 and 0.4 mM ascorbic acid in the above-mentioned buf-

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fer. After incubation at 37°C in a shaking water bath, the malonaldehyde formed was estimated by using the modified thiobarbituric acid method [8]. Values were corrected for endogenous malonaldehyde content.

2.3. *Factors related to lipid peroxidation*

Standard methods, as cited in [8], were used for the estimations of NADPH-cytochrome *c* reductase, cytochrome P-450, phospholipid, degree of unsaturation in lipids, ascorbic acid, α-tocopherol and reduced glutathione. Superoxide dismutase was assayed according to Reiss and Gershon [9]. Cholesterol was estimated as in [10].

3. RESULTS

3.1. *Time course of ascorbate- and NADPH-induced lipid peroxidation*

Figs 1 and 2 show that microsomal fractions from newborn rats are highly resistant to ascorbate- and NADPH-induced lipid peroxidation, respectively. Malonaldehyde production commences only after 10 min incubation in the NADPH-induced system in both fractions of newborn rats, and after 5 min in the ascorbate-induced system in the rough microsomes. In the ascorbate-induced system, most of the malonaldehyde production occurs within 30 min of incubation in both age groups. Smooth microsomes from both age groups are more sensitive to ascorbate-induced lipid peroxidation as compared to rough microsomes. In the case of NADPH-induced lipid peroxidation this differential response is more pronounced in adult rats.

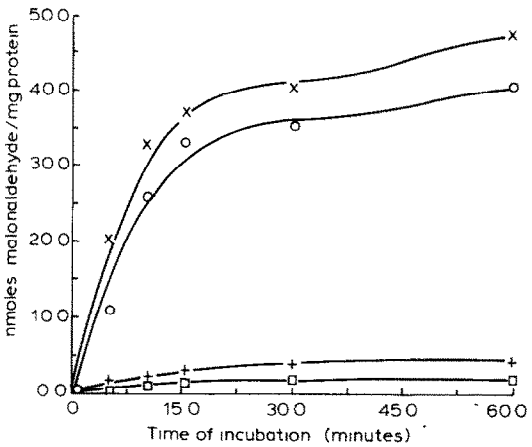

Fig.1. Time course of ascorbate-induced lipid peroxidation in newborn and adult rats. Incubations were carried out as described in section 2 using full complement of cofactors for ascorbate-induced lipid peroxidation with microsomes corresponding to 0.25 mg protein. Each value represents mean ± SE from 5 (adults) or 5 batches (newborns) of rats. (w) Rough and (+) smooth microsomes of newborn rats; (o) rough and (x) smooth microsomes of adult rats.

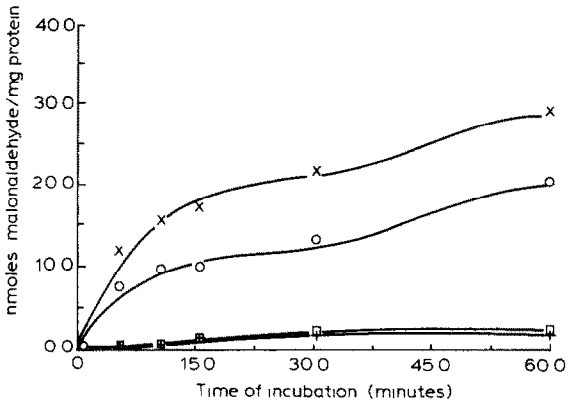

Fig.2. Time course of NADPH-induced lipid peroxidation in newborn and adult rats. Incubations were carried out as described in section 2 using full complement of cofactors for NADPH-induced lipid peroxidation. Other data, as in fig.1.

3.2. *Effect of high concentrations of cofactors on lipid peroxidation*

Data related to the effect of high concentrations of cofactors on lipid peroxidation in rough and smooth microsomes of newborn and adult rats are given in table 1. Ascorbate-induced lipid peroxidation shows a significant increase with 1 mM ascorbate in both age groups. However, at a higher concentration of 4 mM, ascorbate inhibits lipid peroxidation in adults but not in newborn rats. A high concentration of Fe^{2+} (1 mM) inhibits lipid peroxidation in both newborn and adult rats. In the NADPH-induced system, high NADPH concentration (4 mM) increases lipid peroxidation in adult rats but fails to show an effect in the newborns. At a high concentration of 1 mM, Fe^{3+} increases lipid peroxidation in both age groups. Although optimum concentrations of Fe^{2+}, ascorbate and Fe^{3+} significantly increase lipid peroxida-

Table 1

Effect of concentration of cofactors on ascorbate- and NADPH-induced lipid peroxidation in microsomal fractions of newborn and adult rats

System	Cofactor	Concentration (mM)	Lipid peroxidation			
			Rough microsomes		Smooth microsomes	
			Newborn	Adult	Newborn	Adult
Ascorbate-induced	ascorbate	0.40	1.56 ± 0.08	25.50 ± 1.82	2.69 ± 0.08	30.17 ± 1.42
		1.00	2.26 ± 0.14	30.17 ± 2.63	2.89 ± 0.19	38.67 ± 2.56
		4.00	2.96 ± 0.13	18.46 ± 0.61	3.36 ± 0.24	19.36 ± 1.10
	Fe^{2+}	0.05	1.66 ± 0.05	22.52 ± 1.78	3.20 ± 0.15	30.07 ± 0.78
		0.25	2.07 ± 0.06	37.02 ± 0.92	4.34 ± 0.25	42.83 ± 0.86
		1.00	0.89 ± 0.12	18.26 ± 0.48	2.81 ± 0.07	17.50 ± 1.12
NADPH-induced	NADPH	0.40	1.25 ± 0.25	11.82 ± 1.05	0.93 ± 0.07	19.05 ± 2.18
		4.00	1.22 ± 0.18	18.68 ± 2.02	1.10 ± 0.18	32.81 ± 2.32
	Fe^{3+}	0.05	1.35 ± 0.13	10.98 ± 1.56	1.09 ± 0.20	18.75 ± 2.20
		1.00	3.69 ± 0.32	40.22 ± 3.75	5.10 ± 0.61	76.42 ± 8.18

Values are means ± SE from 5 (adults) or 5 batches (newborns) of rats and are expressed as nmol malonaldehyde/mg protein after incubation at 37°C for 15 min. The incubation mixtures contained the full complement of cofactors as described in section 2 except for the cofactor whose concentration is mentioned above

Table 2

Factors related to lipid peroxidation in hepatic rough and smooth microsomes of newborn and adult rats

Factor	Rough microsomes		Smooth microsomes	
	Newborn	Adult	Newborn	Adult
Phospholipid[a]	50 ± 8	260 ± 30[g]	160 ± 20	362 ± 12[g]
Degree of unsaturation in lipids[b]	0.19 ± 0.03	0.93 ± 0.06[g]	0.70 ± 0.08	1.51 ± 0.17[g]
Cholesterol:phospholipid ratio	0.77	0.13	1.00	0.18
NADPH-cytochrome *c* reductase[c]	5.60 ± 0.31	14.82 ± 1.85[g]	6.50 ± 0.38	21.02 ± 0.95[g]
Cytochrome P-450[d]	0.95 ± 0.06	1.53 ± 0.11[g]	0.51 ± 0.05	2.02 ± 0.36[f]
Ascorbic acid[a]	1.46 ± 0.22	0.65 ± 0.10[f]	2.66 ± 0.27	1.74 ± 0.29[e]
Reduced glutathione[a]	5.80 ± 0.82	1.51 ± 0.32[g]	9.85 ± 1.10	4.68 ± 0.72[g]
α-Tocopherol[a]	2.38 ± 0.26	3.06 ± 0.34	2.04 ± 0.22	2.70 ± 0.25
Superoxide dismutase[c]	5.15 ± 0.80	10.02 ± 0.52[g]	3.62 ± 0.61	9.72 ± 0.80[g]

Values are means ± SE from 5 (adults) or 5 batches (newborns) of rats and are expressed as [a] μg/mg protein, [b] μmol equiv./mg protein, [c] units/mg protein and [d] nmol/mg protein. [e] $P < 0.05$, [f] $P < 0.01$ and [g] $P < 0.001$, vs newborn rats

tion in both newborns and adults, lipid peroxidation observed in newborn rats is much less compared with that of adult rats.

3.3. *Factors related to lipid peroxidation in microsomal fractions*

Table 2 presents data on factors related to lipid peroxidation in the microsomal fractions of newborn and adult rats. Factors which enhance lipid peroxidation such as phospholipid content, degree of unsaturation in lipids and activities of NADPH-cytochrome *c* reductase and cytochrome P-450 are lower in the microsomal fractions of newborn rats than in those of adult rats. The

cholesterol:phospholipid ratio, a factor which significantly influences the extent of lipid peroxidation in membranes, is higher in newborn rats. Levels of antioxidants such as glutathione and ascorbic acid are significantly higher in the microsomal fractions of newborn rats. Superoxide dismutase is higher in microsomal fractions of adult rats. The amount of α-tocopherol is similar in both groups. Rough and smooth microsomes significantly differ from each other in their content of most of these factors related to lipid peroxidation in both age groups.

4. DISCUSSION

In rat hepatic endoplasmic reticulum, the enzymatic NADPH-induced and non-enzymatic ascorbate-induced systems are two of the major systems which induce lipid peroxidation [11,12]. Player et al. [13] have reported age-related changes in NADPH-induced lipid peroxidadtion in rat hepatic total microsomes. Since total microsomes contain other organelles besides rough and smooth endoplasmic reticulum [14], age-related studies using total microsomes are not truly representative of changes in the endoplasmic reticulum [7,15]. Besides, our studies have shown that the two microsomal fractions significantly differ from each other in their capacity to undergo lipid peroxidation [8] and in their response to aging [7,16,17]. The present report shows that this is also true with lipid peroxidation in newborn rats.

Previous studies have shown high levels of lipid peroxidation in newborn animals [3,5], however the present study shows that purified hepatic microsomal fractions of newborn rats are highly resistant to lipid peroxidation. The sudden exposure of the neonate to oxygen immediately after parturition as well as the lack of defences against oxygen toxicity has been postulated as the major cause of the observed high levels of lipid peroxidation in newborns [6]. The present study shows that the resistance observed in the microsomal fractions of newborn rats is not due to high levels of superoxide dismutase, one of the major defences against oxygen toxicity [2,18].

Lipid peroxidation in biological membranes is regulated by the interaction of several factors [2,18]. The main substrates for lipid peroxidation are the polyunsaturated fatty acids, mainly present in the phospholipids [2,19]. Ascorbate, Fe^{2+}, oxygen and functioning of the electron transport chains increase lipid peroxidation. Antioxidants such as glutathione, α-tocopherol and inhibitors of free radical reactions like superoxide dismutase inhibit lipid peroxidation [2,18]. Besides, the physical state of membrane lipids including fluidity and surface charge also regulates the extent of lipid peroxidation in membranes [2,18,21].

This study shows that microsomal fractions of newborn rats contain lower amounts of substrate as shown by the level of phospholipid and degree of unsaturation in lipids. Other factors which enhance lipid peroxidation such as cytochrome P-450, the key component of the microsomal electron transport chain [2,7,18], and NADPH-cytochrome *c* reductase, which mediates NADPH-induced lipid peroxidation [20], are lower in the microsomes of newborn rats. The lack of increase observed with a high concentration of NADPH in the newborn rats may be mainly because of this low amount of NADPH-cytochrome *c* reductase. Glutathione, a potent inhibitor of microsomal lipid peroxidation [22,23], is present in greater amounts in the newborn rats. Microsomal fractions of newborn rats have higher cholesterol: phospholipid ratios. This may contribute to the observed resistance to lipid peroxidation in newborn rats by decreasing the fluidity of membranes [21].

The low level of superoxide dismutase, a major defence against oxygen toxicity and lipid peroxidation [2,6], and the high amount of ascorbic acid which stimulates lipid peroxidation [2,18] should have conferred some degree of sensitivity to lipid peroxidation on the microsomal fractions of newborn rats. However, our data indicate a high degree of resistance to lipid peroxidation, involving low availability of substrate, low amount of NADPH-cytochrome *c* reductase and high levels of glutathione as the principal factors responsible. If glutathione is the major factor responsible for resistance, increasing the time of incubation or higher concentration of co-factors should have removed this resistance [22,23]. If NADPH-cytochrome *c* reductase is the key factor, there should not have been any resistance to ascorbate-induced lipid peroxidation. However, the present study shows no such trend. Thus, the observed resistance to lipid peroxidation in the newborn rats

supports the hypothesis that the rate-limiting step of lipid peroxidation is substrate availability [21,24]. Since the proportion of lipid peroxidation observed in newborns to adults, as compared to the proportion of phospholipid and degree of unsaturation in lipids of these age groups, is still low, the physical state of membrane lipids as determined by the cholesterol:phospholipid ratio appears to play a major role in the resistance of microsomes of newborn rats to lipid peroxidation.

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