J. Steroid Biochem. Molec. Biol. Vol. 42, No. 7, pp. 729–736, 1992
Printed in Great Britain

0960-0760/92 $5.00 + 0.00
Pergamon Press Ltd

THE INFLUENCE OF NADPH-DEPENDENT LIPID PEROXIDATION ON THE PROGESTERONE BIOSYNTHESIS IN HUMAN PLACENTAL MITOCHONDRIA

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(Received 29 January 1992)

Summary—In an *in vitro* system consisting of human term placental mitochondria and an NADPH-generating system plus Fe^{2+}, significant lipid peroxidation was observed along with a concomitant inhibition of progesterone biosynthesis. This inhibition could be markedly blocked by Mn^{2+}, superoxide dismutase and dimethylfuran, inhibitors of NADPH-dependent lipid peroxidation. In addition, it has been found that malondialdehyde formation is accompanied by a corresponding decrease in placental mitochondrial cytochrome *P*-450 content. Inhibitors of lipid peroxidation also prevent the loss of cytochrome *P*-450, further demonstrating a direct relationship between NADPH-dependent lipid peroxidation and degradation of cytochrome *P*-450 in cell-free systems. These measurements provide the first evidence that the inhibition of progesterone biosynthesis by a NADPH-dependent lipid peroxidation in placental mitochondria is a consequence of cytochrome *P*-450 degradation due to lipid peroxidation.

INTRODUCTION

Lipid peroxidation has been widely investigated as a possible mediator of various pathological and physiological processes [1–3]. Among the apparent effects of lipid peroxidation in the liver are changes in the activities of microsomal enzymes; the cytochrome *P*-450-containing monooxygenases are especially vulnerable [4–6]. Levin *et al.* [7] showed in rat liver microsomes, a direct relationship between the loss of cytochrome *P*-450 heme groups, the loss of microsomal polyunsaturated fatty acids and the formation of malondialdehyde, the end product of lipid peroxidation.

Although the relationship between lipid peroxidation and monooxygenase activities has been extensively studied in hepatic microsomes, little is known about the consequences of lipid peroxidation on enzymes in extrahepatic tissues. High levels of lipid peroxidation have been demonstrated in adrenocortical mitochondrial and microsomal preparations [8–12]. It is known that lipid peroxidation can effect the degradation of various adrenal steroidogenic enzymes, apparently by destruction of cytochrome *P*-450 [8, 11–13]. Thus, it has been proposed that lipid peroxidation may play a role in the modulation of steroidogenesis [3].

Human placental mitochondria also contain cytochrome *P*-450, which is a component of the NADPH–cytochrome *P*-450-linked monooxygenase system. This electron-transfer system being responsible for the cleavage of cholesterol side-chain in the course of progesterone biosynthesis, consists of flavoprotein and an iron–sulfur protein (NADPH–cytochrome *P*-450 reductase), and cytochrome *P*-450 [14]. As shown in the previous papers [15, 16] the placental mitochondria appear to be very susceptible to NADPH-dependent lipid peroxidation. The present work was undertaken to study the effects of NADPH-dependent lipid peroxidation on progesterone biosynthesis in human placental mitochondria.

EXPERIMENTAL

Materials

$NADP^+$, glucose-6-phosphate, 2,5-dimethylfuran, cytochrome *c*, thiobarbituric acid, superoxide dismutase and glucose-6-phosphate dehydrogenase were purchased from Sigma (St Louis, MO), $MnSO_4$ "AnalaR" from BDH Chemicals Ltd (England), $FeCl_2$ from E. Merck (Darmstadt, Germany), [4-^{14}C]cholesterol (58 mCi/mmol), [4-^{14}C]pregnenolone (55 mCi/

mmol), [^{3}H]progesterone (12 Ci/mmol) and [^{3}H]pregnenolone (6.9 Ci/mmol) were products of Radiochemical Centre (Amersham, England). All other materials were of highest analytical grade available from POCh (Gliwice, Poland).

Preparation of placental mitochondria

Human term placental mitochondria were prepared as described previously [15].

Extraction, purification and assay of progesterone

The incubation mixtures were transferred to conical tubes containing known amounts of [^{3}H]progesterone and [^{3}H]pregnenolone which were used to check recovery. After completing the incubations, non-radioactive progesterone and pregnenolone (2 mg of each steroid) were also added as unlabeled carrier. The contents of tubes were extracted twice with 10 ml of chloroform–diethyl ether (1:5, v/v). The organic and aqueous phases were separated by centrifugation and the organic phase was evaporated to dryness. The dry residue was subjected to thin-layer chromatography on Silica Gel G (Merck) impregnated with Rhodamine 6G. On developing the chromatogram in (I) methylene chloride–diethyl ether (5:2, v/v), three fractions were obtained: progesterone, pregnenolone and cholesterol. Progesterone and pregnenolone fractions were further purified by thin-layer chromatography in the following systems: (II) benzene–ethanol (9:1, v/v), (III) benzene–ethyl acetate (3:2, v/v) and (IV) methylene chloride–methanol (98:2, v/v). Purification was continued to a constant $^{14}C/^3H$ ratio in the end products.

The radioactivity of isolated steroid was measured with an efficiency of 36% for ^{3}H and 61% for ^{14}C in 10 ml of scintillation fluid containing 4 g of 2,5-diphenyloxazole and 0.2 g of 1,4-di-2(5-phenyloxazolyl)benzene per liter of toluene, using a Nuclear Chicago Isocap 300 liquid scintillation spectrometer. Total incorporation of ^{14}C into [^{14}C]progesterone was found on the basis of tritium found in the final product of purification procedure.

Other analytical procedures

Lipid peroxidation was determined by the formation of thiobarbituric acid-reactive substances (TBARS) as described previously [16]. Values for the TBARS are expressed in nmol malondialdehyde (MDA) per mg mitochondrial protein using a molar absorption coefficient of $1.56 \times 10^5\ M^{-1} \times cm^{-1}$ at 535 nm [17].

Mitochondrial NADPH–cytochrome *P*-450 reductase activity was assayed as reported previously [16] using a molar absorption coefficient of $21 \times 10^3\ M^{-1} \times cm^{-1}$ for the reduced cytochrome *c* at 550 nm.

Cytochrome *P*-450 was determined by a modification of the method described by Omura and Sato [18] using a molar absorption coefficient of $100 \times 10^3\ M^{-1} \times cm^{-1}$ [19] for the difference in absorbance between 450 and 490 nm. Mitochondrial suspensions in 50 mM phosphate buffer (pH 7.4) containing known amounts of protein (3 mg/ml) were bubbled with CO for 1 min after which 2.5 ml of this solution was transferred to the sample and reference cuvettes and a base-line was determined. After addition of a few grains of sodium dithionite to the sample cuvette a difference spectrum was obtained. In this way, the contribution of hemoglobin was balanced out in each cuvette due to formation of the CO complex. Optical spectroscopy was carried out in a 3-ml optical cell of 1-cm light path at room temperature using a Hitachi model 356 (Tokyo, Japan) two wavelength, double beam spectrophotometer.

Protein was determined by the biuret method [20] in 0.25% sodium deoxycholate with bovine serum albumin as the standard.

RESULTS

The effect of NADPH-dependent lipid peroxidation on progesterone biosynthesis

Figure 1 shows the relationship between progesterone biosynthesis and MDA formation. NADPH-dependent lipid peroxidation which increased rapidly with the increasing Fe^{2+} concentration to about 0.1 mM was accompanied by the inhibition of progesterone biosynthesis from cholesterol under the conditions employed. As in the previous experiments [21, 22] pregnenolone, a generally accepted intermediate of progesterone biosynthesis, did not accumulate to any significant extent after 30 min incubation. Formation of progesterone from cholesterol in the human placental mitochondrial fraction involves the side-chain cleavage of cholesterol to pregnenolone and a subsequent conversion of pregnenolone to progesterone [23]. Therefore, the effect of NADPH-dependent lipid peroxidation on the latter

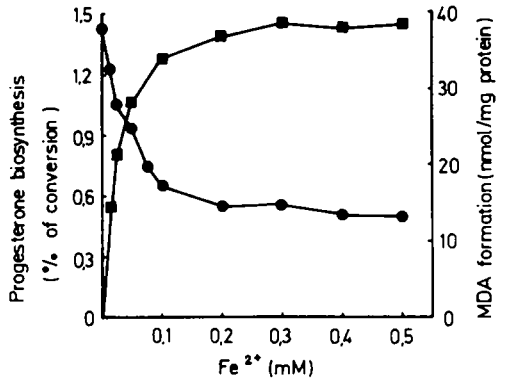

Fig. 1. The effect of NADPH-dependent lipid peroxidation induced by increasing concentrations of Fe^{2+} on progesterone biosynthesis from cholesterol in placental mitochondria. The incubation was carried out for 30 min at 37°C under aerobic conditions with constant shaking in 2.5 ml medium containing 0.1 M Tris–HCl buffer (pH 7.4), 2 mg of mitochondrial protein, 0.25 μCi [4-^{14}C]cholesterol and an NADPH-generating system consisting of 0.5 mM $NADP^+$, 3 mM glucose-6-phosphate and 5 U of glucose-6-phosphate dehydrogenase (1 U = 1 μmol of glucose-6-phosphate oxidized per min at pH 7.4 at 30°C in the presence of $NADP^+$). Various concentrations of $FeCl_2$ were added to the reaction mixture as indicated in the figure. Assays were performed as described in Experimental. ■, MDA formation; ●, conversion of [4-^{14}C]cholesterol to [4-^{14}C]progesterone. The values are means from five experiments.

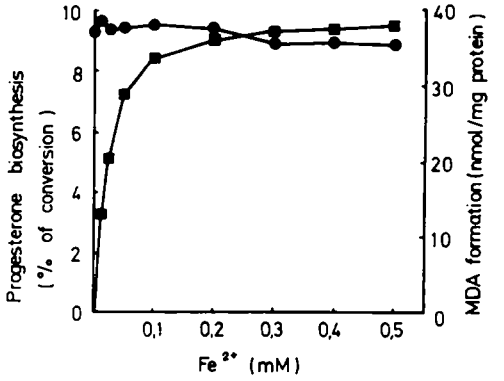

Fig. 2. The effect of NADPH-dependent lipid peroxidation induced by increasing concentrations of Fe^{2+} on progesterone biosynthesis from pregnenolone in placental mitochondria. The experimental conditions were the same as described in Fig. 1, except that 0.25 μCi [4-^{14}C]pregnenolone with 20 μg of cold pregnenolone was used as a substrate for progesterone biosynthesis. ■, MDA formation; ●, conversion of [4-^{14}C]pregnenolone to [4-^{14}C]-progesterone. The values are means from four experiments.

conversion was investigated. As shown in Fig. 2, in contrast to progesterone from cholesterol formation, the conversion of pregnenolone to progesterone was not affected by NADPH-dependent lipid peroxidation. This implies that the inhibition of progesterone from cholesterol biosynthesis by NADPH-dependent lipid peroxidation should be ascribed to its effect on the cytochrome *P*-450-containing mixed-function oxidase.

The effect of the NADPH-dependent lipid peroxidation inhibitors on progesterone biosynthesis

Various compounds have been shown to inhibit NADPH-dependent lipid peroxidation in human placental mitochondria [15]. Several of these compounds were tested for their ability to prevent the inhibition of progesterone biosynthesis during NADPH-dependent lipid peroxidation. As shown in Table 1, the increase of MDA formation was associated with decreased progesterone biosynthesis. This inhibition could be reversed by Mn^{2+}, superoxide dismutase and dimethylfuran, each being a very effective inhibitor of NADPH-dependent lipid peroxidation. The progesterone biosynthesis was not affected by these inhibitors in the absence of lipid peroxidation. The data of these experiments clearly indicate that NADPH-dependent lipid peroxidation may cause a decrease of the rate of progesterone biosynthesis in human placental mitochondria.

Comparison of the effects of NADPH-dependent lipid peroxidation on progesterone biosynthesis, NADPH–cytochrome P-450 reductase activity and cytochrome P-450 content

As shown in Table 2, every decrease of progesterone biosynthesis produced by NADPH-

Table 1. The effect of NADPH-dependent lipid peroxidation inhibitors on progesterone biosynthesis in placental mitochondria

Additions	MDA formation (nmol/mg protein)	Progesterone biosynthesis	
		dpm ^{14}C per sample	% of conversion
NADPH-generating system without Fe^{2+}	0.0 ± 0.0	8500 ± 700	1.5
+50 μM Mn^{2+}	0.0 ± 0.0	9600 ± 1200	1.7
+100 U/ml superoxide dismutase	0.0 ± 0.0	9100 ± 900	1.6
+1 mM dimethylfuran	0.0 ± 0.0	8800 ± 1100	1.6
NADPH-generating system with Fe^{2+}	31.5 ± 4.2	4700 ± 600	0.85
+50 μM Mn^{2+}	0.0 ± 0.0	9900 ± 800	1.8
+100 U/ml superoxide dismutase	8.4 ± 0.9	7600 ± 600	1.4
+1 mM dimethylfuran	9.1 ± 0.4	7800 ± 1000	1.4

The experimental conditions were the same as described in Fig. 1 except that 0.1 mM $FeCl_2$ was added to the reaction mixture where indicated. Final concentration of $MnSO_4$, dimethylfuran and superoxide dismutase as indicated. The results are expressed as mean ± SD from four experiments. MDA = malondialdehyde.

Table 2. The effects of NADPH-dependent lipid peroxidation on progesterone biosynthesis, NADPH–cytochrome *P*-450 reductase activity and cytochrome *P*-450 content in human placental mitochondria

Additions	MDA formation (nmol/mg protein)	Progesterone biosynthesis: dpm ^{14}C per 2.5 ml of sample	Progesterone biosynthesis: % of conversion	NADPH-cyt *P*-450 reductase activity (nmol/min/mg protein)	Cytochrome *P*-450 content (pmol/mg protein)
Control	0.0 ± 0.0	0 ± 0	0	12.5 ± 0.6	116 ± 9
+ 12.5 μM Fe^{2+}	0.9 ± 0.1	0 ± 0	0	13.1 ± 1.4	118 ± 7
+ 100 μM Fe^{2+}	2.3 ± 0.3	0 ± 0	0	12.9 ± 1.2	112 ± 12
+ NADPH-generating system	0.0 ± 0.0	7900 ± 900	1.4	12.7 ± 1.1	120 ± 5
+ NADPH-generating system + 12.5 μM Fe^{2+}	13.8 ± 1.5	6600 ± 400	1.2	12.0 ± 0.9	92 ± 8
+ NADPH-generating system + 100 μM Fe^{2+}	33.1 ± 5.2	3800 ± 600	0.67	11.4 ± 1.5	50 ± 6

The incubation was carried out for 30 min at 37°C in 50 ml medium containing 0.1 M Tris–HCl buffer (pH 7.4), 40 mg of mitochondrial protein and 5 μCi of [4-^{14}C]cholesterol. The NADPH-generating system consisting of 0.5 mM $NADP^+$, 3 mM glucose-6-phosphate and 100 U of glucose-6-phosphate dehydrogenase was added to the reaction mixture where indicated. The lipid peroxidation was terminated by adding 1 mM EDTA. 0.5 ml and 2.5 ml of the samples were taken for MDA and progesterone determination, respectively. All samples were centrifuged at 10,000 *g* for 15 min. Then, the mitochondria were washed twice in the medium containing 0.32 M mannitol, 10 mM Tris–HCl buffer (pH 7.4) and 1 mM EDTA. The washed mitochondria were used for NADPH–cytochrome *P*-450 reductase activity and cytochrome *P*-450 content determination. Assays were performed as described in Experimental. The results were expressed as mean ± SD from four to eight experiments.

dependent lipid peroxidation induced by increasing concentrations of Fe^{2+}, was accompanied by a significant loss of cytochrome *P*-450, while NADPH–cytochrome *P*-450 reductase activity was virtually unchanged under these conditions. Therefore, the decrease in progesterone biosynthesis during the NADPH-dependent lipid peroxidation seems to be caused mainly by the reduction of cytochrome *P*-450 concentration, but not by the change of the NADPH–cytochrome *P*-450 reductase activity. The lipid peroxidation may either be directly responsible for the destruction of cytochrome *P*-450 or the relationship may be merely coincidental. It is possible that Fe^{2+} used to initiate lipid peroxidation has a direct effect on cytochrome *P*-450 degradation. Thus, the effect of Fe^{2+} alone on cytochrome *P*-450 content was investigated. As shown in Table 2, Fe^{2+} displayed no effect on cytochrome *P*-450 content in the absence of a significant lipid peroxidation.

To determine whether the main product of lipid peroxidation, MDA, might be responsible for any of the effects observed, mitochondria were incubated either in the presence or in the absence of exogenous MDA. The addition of MDA (50 nmol/mg mitochondrial protein) to the incubation medium, containing the NADPH-generating system alone, did not change either the cytochrome *P*-450 content or the progesterone biosynthesis (data not shown).

Relation between the loss of cytochrome P-450 and NADPH-dependent lipid peroxidation

Figure 3 shows the time-dependent relationship between cytochrome *P*-450 content and MDA formation. NADPH-dependent lipid peroxidation was linear up to 30 min. Maximum levels of NADPH-dependent lipid peroxidation were observed after 40 min. Throughout the incubation time, a close correlation between the MDA formation and a concomitant loss of cytochrome *P*-450 content was observed. In the absence of lipid peroxidation very little degradation of cytochrome *P*-450 occurred. Unincubated mitochondria contained 126 ± 13 pmol of cytochrome *P*-450 per mg protein. Incubation of placental mitochondria for 60 min with the NADPH-generating system plus Fe^{2+} (peroxidizing medium) resulted in a 70% decrease of cytochrome *P*-450 content whereas the cytochrome *P*-450 loss during the incubation in the absence of both the NADPH-generating system and Fe^{2+} (non-peroxidizing medium) was shown to be 9% only. Additions of Fe^{2+} or

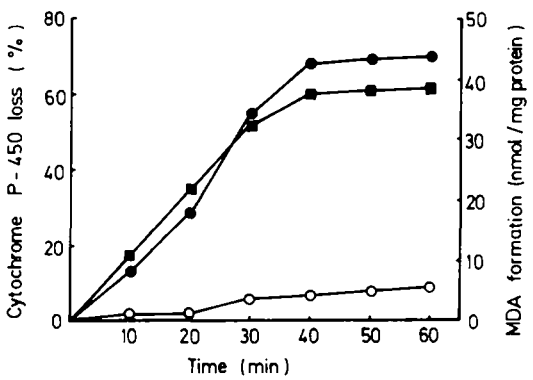

Fig. 3. The time-dependent effect of NADPH-dependent lipid peroxidation on cytochrome *P*-450 degradation. The experimental conditions were the same as described in Table 2, except that [4-^{14}C]cholesterol was omitted and the NADPH-generating system and 100 μM $FeCl_2$ were present in the incubation medium. ■, MDA formation; ●, cytochrome *P*-450 loss; ○, cytochrome *P*-450 loss in the absence of the NADPH-generating system and Fe^{2+} (control). The values are means from six experiments.

the NADPH-generating system alone to non-peroxidizing medium caused no significant change of cytochrome *P*-450 loss (data not shown).

Additional evidence for the role of NADPH-dependent lipid peroxidation in cytochrome *P*-450 degradation has been provided by the results of experiments in which Mn^{2+} or EDTA, known inhibitors of NADPH-dependent lipid peroxidation [7, 15, 24] had been added. As shown in Table 3, the additions of Mn^{2+} and EDTA to peroxidizing medium completely blocked both, the NADPH-dependent lipid peroxidation and the loss of cytochrome *P*-450 content due to lipid peroxidation. The presence of either Mn^{2+} or EDTA in the non-peroxidizing medium resulted in no significant changes in the cytochrome *P*-450 content (data not shown).

In summary, these studies demonstrate an excellent correlation between the loss of cytochrome *P*-450 and NADPH-dependent lipid peroxidation in human placental mitochondria.

Relation between NADPH-dependent lipid peroxidation induced losses of cytochrome P-450 and progesterone biosynthesis

From the results presented above one may conclude, that NADPH-dependent lipid peroxidation is accompanied by a loss of cytochrome *P*-450, which causes a decrease of progesterone biosynthesis. To confirm this assumption, an additional experiment was performed. Mitochondria were preincubated in a peroxidizing and a non-peroxidizing medium. Also the medium in which Fe^{2+} or the NADPH-generating system were omitted, was used as a control. Peroxidation was stopped by addition of 1 mM EDTA to the preincubation mixture. The mitochondria were then washed and used for the main incubation in which progesterone biosynthesis was measured. The amount of intact cytochrome *P*-450 in the peroxidized placental mitochondria was about 46% of the control non-peroxidized mitochondria. The results presented in Fig. 4 indicate that progesterone biosynthesis was dependent upon the amount of cytochrome *P*-450 present in placental mitochondria. In the peroxidized placental mitochondria the progesterone biosynthesis was about 50% lower than in the non-peroxidized mitochondria. These results strongly support the idea that the inhibition of progesterone biosynthesis by a NADPH-dependent lipid peroxidation in placental mitochondria is a consequence of cytochrome *P*-450 degradation due to lipid peroxidation.

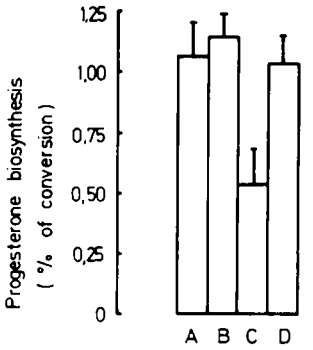

Fig. 4. The effect of lipid peroxidation-induced losses of mitochondrial cytochrome *P*-450 on progesterone biosynthesis. The mitochondria were preincubated without the NADPH-generating system and $FeCl_2$ (A); with the NADPH-generating system (B); with the NADPH-generating system plus 100 μM $FeCl_2$ (C) and with 100 μM $FeCl_2$ alone (D). The experimental conditions were the same as those described in Table 2, except that [4-^{14}C]cholesterol was omitted. After 30 min preincubation, the lipid peroxidation was terminated by adding 1 mM EDTA. All samples were centrifuged and mitochondrial pellet was washed as described in Table 2. One part of washed mitochondria was resuspended in 10 mM phosphate buffer (pH 7.4) and used for cytochrome *P*-450 determination. The remaining mitochondria were used to the next incubation in which conversion of [4-^{14}C]cholesterol to [4-^{14}C]progesterone was measured. The experimental conditions were the same as described in Fig. 1, except that Fe^{2+} was omitted. The mitochondrial contents of cytochrome *P*-450 before the incubation were: (A) 116 ± 10; (B) 121 ± 13; (C) 53 ± 8 and (D) 114 ± 15 pmol of cytochrome *P*-450 per mg mitochondrial protein. The results are the mean ± SD of five experiments expressed as percent of [4-^{14}C]cholesterol to [4-^{14}C]progesterone conversion.

Table 3. The effects of inhibitors of NADPH-dependent lipid peroxidation on cytochrome *P*-450 content

Conditions of incubation	MDA formation (nmol/mg protein)	Cytochrome *P*-450 content (pmol/mg protein)
Mitochondria incubated without NADPH-generating system and Fe^{2+} (control)	0.0 ± 0.0	118 ± 11
Mitochondria incubated with NADPH-generating system + 100 μM Fe^{2+}	32.7 ± 3.8	54 ± 7
+ 50 μM Mn^{2+}	0.0 ± 0.0	111 ± 8
+ 1 mM EDTA	0.0 ± 0.0	114 ± 12

The experimental conditions were the same as described in Table 2. Final concentrations of $FeCl_2$, $MnSO_4$ and EDTA as indicated. The results are expressed as mean ± SD from four experiments.

DISCUSSION

Low levels of lipid peroxidation are essential to many normal cellular processes [25] and occur in a number of tissues including placenta [26]. Although a variety of antioxidant mechanisms serve to control lipid peroxidation, under certain conditions the protective mechanism can be overwhelmed, leading to elevated steady-state tissue levels of peroxidation products [27, 28]. Conditions which can stimulate lipid peroxidation are numerous and include hyperoxia, hypoxia, iron toxicity and antioxidant deficiencies [29–32]. This uncontrolled lipid peroxidation in biological membranes would result in damage of the membrane structure and thus of various cellular functions observed both *in vitro* and *in vivo* [33, 34]. One of the most important lipid peroxidation consequences is a significant decrease in the activity of several membrane-bound microsomal and mitochondrial enzymes [33, 35–37].

Kitabchi [38, 39] showed a direct relationship between lipid peroxidation and a decline in adrenal steroid hydroxylase activities. Furthermore, numerous studies have demonstrated that lipid peroxidation can affect the degradation of cytochrome *P*-450 in adrenocortical microsomal and mitochondrial preparations [8, 11–13, 40]. So far, however, nothing was known about the effect of lipid peroxidation on the cytochrome *P*-450-containing monooxygenase system required for progesterone biosynthesis in human placental mitochondria. As shown in earlier studies [15, 16] the placental mitochondria appear to be very susceptible to NADPH-dependent lipid peroxidation. The present investigation shows for the first time that placental mitochondrial NADPH-dependent lipid peroxidation may be responsible at least *in vitro* for a decline in progesterone biosynthesis. Moreover, the present results indicate unequivocally that the inhibition of progesterone biosynthesis by a NADPH-dependent lipid peroxidation is a consequence of cytochrome *P*-450 degradation. This notion is supported by the following evidence provided: (1) the NADPH-dependent lipid peroxidation induced by increasing concentrations of Fe^{2+} strongly inhibited progesterone from cholesterol biosynthesis being without any effect on progesterone biosynthesis from pregnenolone; (2) the inhibitors of NADPH-dependent lipid peroxidation: Mn^{2+}, superoxide dismutase and dimethylfuran, were strongly relieving the progesterone biosynthesis inhibition due to lipid peroxidation; (3) the decrease in progesterone biosynthesis produced by NADPH-dependent lipid peroxidation was found to be accompanied by a significant loss of cytochrome *P*-450; (4) time-dependent production of MDA paralleled the loss of cytochrome *P*-450; (5) inhibitors of NADPH-dependent lipid peroxidation also prevented the loss of cytochrome *P*-450; and (6) the progesterone biosynthesis was lower in the mitochondria containing decreased levels of cytochrome *P*-450.

Recently, it has been suggested that superoxide anion liberated from cytochrome *P*-450, in combination with iron, may be responsible for the initiation of NADPH-dependent lipid peroxidation in human placental mitochondria [16]. On the other hand, the present study indicates that cytochrome *P*-450 is degraded during NADPH-dependent lipid peroxidation. Thus, it is possible that the superoxide anion generated by cytochrome *P*-450 could initiate peroxidation of lipids with subsequent degradation of placental mitochondrial cytochrome *P*-450. These suggestions agree with those of Hornsby and Crivello [3], who reported that cytochrome *P*-450 in adrenocortical cells have a unique relationship with lipid peroxidation, both causing the initiation of lipid peroxidation and being targets for inactivation by the same process.

It is known that in steroidogenic tissues such as adrenal cortex, lipid peroxidation destroys various forms of cytochrome *P*-450 [3]. The activity of microsomal 17α-hydroxylase, 21-hydroxylase and cytochrome *P*-450 (all being involved in adrenocortical steroidogenesis) are lowered by lipid peroxidation [13, 41]. Similarly, Klimek *et al.* [11] have found that NADPH-dependent lipid peroxidation promoted degradation of cytochrome $P\text{-}450_{11\beta}$ as well as cytochrome $P\text{-}450_{scc}$ in bovine adrenocortical mitochondria. However, the cytochrome $P\text{-}450_{scc}$ was only slightly affected by NADPH-dependent lipid peroxidation, the cytochrome $P\text{-}450_{11\beta}$ being much more susceptible to this process. It is well known that in human placental mitochondria all of the cytochrome *P*-450 is involved in cholesterol side-chain cleavage [42]. The present findings have demonstrated that, under conditions employed, the placental mitochondrial cytochrome *P*-450 is strongly degradated by NADPH-dependent lipid peroxidation. Thus, it is reasonable to think that placental mitochondrial cytochrome $P\text{-}450_{scc}$ is

more susceptible to this process than adrenocortical mitochondrial cytochrome $P\text{-}450_{scc}$. However, it is worthwhile to note, that the absolute level of cytochrome P-450 in placental mitochondria (0.126 nmol/mg protein) is considerably lower than in adrenocortical mitochondria, in which cytochrome P-450 is found in concentrations of 1.25–1.43 nmol/mg protein [11].

In contrast to cytochrome P-450 degradation, NADPH–cytochrome P-450 reductase activity in placental mitochondria was not influenced by NADPH-dependent lipid peroxidation. These results agree with those of Imataca *et al.* [13], who observed that the activity of NADPH–cytochrome P-450 reductase remained unchanged in porcine adrenocortical microsomes, whereas the content of cytochrome P-450 was apparently decreased while lipid peroxidation was increased. On the other hand, these authors observed a decrease of pregnenolone to progesterone conversion during the Fe^{2+}-induced microsomal lipid peroxidation. In the human placental mitochondria investigated here, NADPH-dependent lipid peroxidation was without significant effect on the conversion of pregnenolone to progesterone. Thus, on the basis of experiments reported here, it seems to be clear that only the degradation of cytochrome P-450 may be responsible for the inhibition of progesterone biosynthesis observed in human placental mitochondria during the NADPH-dependent lipid peroxidation.

Finally, it is suggested that in human placental mitochondria NADPH-dependent lipid peroxidation may play an important role in the modulation of progesterone biosynthesis. Thus, one may assume that physiopathological conditions leading to the mitochondrial lipid peroxidation in human placenta is causing a decrease of progesterone biosynthesis in this tissue. However, physiological significance of these findings remains to be established.

Acknowledgements—I am indebted to Professors L. Żelewski and M. Żydowo for their interest in this work and for reading the manuscript. I also wish to thank S. Czarnecki (M.Sc.), for excellent technical assistance. This work was supported by a grant from the Polish Academy of Sciences, within the project No. 3.13.6.1.8 and a grant from the KBN, within the project St-40.

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