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Regulation of lipid peroxidation in mitochondria by nitroglycerine

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The mechanisms of lipid oxidation under the application of nitroglycerine (NG) were studied in isolated rat heart and liver mitochondria. Dose-dependent formation of diene conjugates (DC), leukotriene C_4 (LTC_4) and thromboxane B_2 (TxB_2) was shown. To disclose the mechanisms regulating lipid peroxidation in mitochondria, we studied the effect of NG application on the formation of prooxidants (H_2O_2 and free Fe^{2+}), as well as xanthine oxidase and mtNOS activity as main sources of ROS and RNS. Based on the correlation dependences, we have found that DC, LTC_4, and TxB_2 formation was strongly dependent on hydroperoxide production and free divalent iron release in mitochondria. Also, DC formation exhibited the dependence on Ca^{2+} uptake in mitochondria. No dependence of lipid oxidation on xanthine oxidase activity was found. In heart, but not liver mitochondria, DC, LTC_4, and TxB_2 exhibited strong dependence on mtNOS activity, but were independent of nitrosothiols formation. This indicated that lipid oxidation was independent of direct protein nitrosylation caused by NG application. No dependence of lipid oxidation on mtNOS activity in liver was found, which agreed with much higher mtNOS activity in heart mitochondria, and suppression of mtNOS activity in liver mitochondria at high doses of NG. So, we came to the conclusion that under NG application ROS overproduction and free Fe^{2+} release promoted both enzymatic and non-enzymatic lipid oxidation in heart and liver mitochondria. Also, we hypothesized that RNS overproduction due to the elevated mtNOS activity in the heart could largely contribute to lipid peroxidation and promote much faster increase in the formation of lipid oxidation products in heart as compared to liver mitochondria, especially at high doses of NG. Obtained correlation dependences allowed us disclose free iron, hydroperoxide, and mtNOS activity as principal factors affecting lipid peroxidation in mitochondria under NG application.
Key words: nitroglycerine; heart; liver; mitochondria; ROS; RNS; lipid peroxidation; mtNOS.

INTRODUCTION

Cardioprotective effects of NO donors occur *via* NO release from the chemical compounds, triggering of NO/cGMP/PKG-dependent pathway, and direct nitrosylation of cellular proteins. [1-3]. Nitroglycerine (NG) is one of most widely used NO donors, which releases NO with the involvement of the enzyme aldehyde dehydrogenase [4]. Mitochondria are one of the key targets in cardioprotective effects of NG, which largely are mediated by the blockage of permeability transition pore (mPTP). In our study we have shown the mPTP blockage by NG administration *in vivo* [5], and literary data have shown that mPTP blockage by NG occurred by direct nitrosylation of critical thiols of cyclophillin D, one of the main regulatory proteins of mPTP [2].

However, the impact of NG application on mitochondrial metabolism remains little known yet. Mitochondria possess their own constitutive Ca^{2+} dependent NOS isoform, so-called mtNOS, and are one of the most important players in cellular NO cycle [6-8]. As we have shown in our works, NG application greatly affected RNS metabolism in mitochondria [5, 9]. Recently we observed inverse regulation of RNS and ROS production in heart and liver mitochondria under the action of NG, i.e. high RNS and low ROS production in heart mitochondria and, *vice versa*, lower RNS formation and much higher ROS

release in liver mitochondria [9]. This motivated us to study the effect of NG application on lipid oxidative metabolism with the aim to compare basic regulatory mechanisms of lipid oxidation in heart and liver mitochondria.

As it is known, lipid oxidation mechanisms include non-enzymatic lipid oxidation by multiple cellular prooxidants, primarily ROS and toxic RNS, such as peroxynitrite [10], and enzymatic oxidation, starting from the activation of phospholipase(s) A_2, release of arachidonic acid (AA) and triggering of arachidonic acid cascade with consequent formation of its end products, eicosanoids (prostaglandins, leukotrienes, thromboxanes, numerous homologs of HETEs and EETs) by the enzymes lipoxygenase, cyclooxygenase and cytochrome P450 [11]. Eicosanoids, especially thromboxanes and leukotrienes,are commonly known markers of lipid oxidation and inflammation, implicated in the development of several diseases, and pathophysiological conditions including heart failure, hypertension, diabetes and cancer [12].

At present, there are still too scarce literary data about pathways of lipid metabolism in mitochondria [13-15]. Till recently, it was generally supposed that biosynthesis of downstream products of PLA_2/AA pathway occurs outside mitochondria, and the existence of PLA_2/AA cascade in mitochondria remained rather hypothetical. For decades it was known that mitochondria possess both Ca^{2+}-dependent and Ca^{2+}-independent isoforms of phospholipase A_2 activated by Ca^{2+} and ROS [13, 14, 16], but the ability of mitochondria to endogenously produce lipid mediators was doubted, and the functions of mitochondrial PLA_2 remained unclear. However, findings of the past decade have shown the production of arachidonic acid and eicosanoids following the activation of mitochondrial PLA_2 by Ca^{2+} ions [13]. Also, it was shown that thromboxanes and leukotrienes formed outside mitochondria upon activation of cytosolic PLA_2 can be transported to mitochondria and affect mitochondrial functions by taking part in β-oxidation [17], by interacting with uncoupling proteins and modulating ROS production [14], and by facilitation of mPTP opening [16].

Based on our recent research showing cell-specific regulation of ROS and RNS production in mitochondria under NG application [9], in this work we studied the dose-dependent effects of NG on the content of diene conjugates (DC) and eicosanoids, leukotriene C_4 (LTC_4) and thromboxane B_2 (TxB_2) in rat heart and liver mitochondria with the aim to disclose basic cell-specific mechanisms regulating lipid oxidation in mitochondria under NG application.

METHODS

All procedures performed in the studies were in accordance with EU directive 86/609/EEC and the ethical standards approved by the Ethics Committee at A.A. Bogomoletz Institute of Physiology, NAS of Ukraine. Adult Wistar rats with 180-200 g body weight were used. Nitroglycerine (NG) was administered intraperitoneally at the doses 0.25, 0.5, 1.0 and 1.5 mg/kg weight. Control animals were administered physiological solution.

Mitochondria were isolated by the standard procedure. Hearts and liver removed at 5th min after NG administration were thoroughly washed with 0,9% KCl (2°C) minced and homogenized in standard isolation medium: 250 mM sucrose, 20 mM Tris-HCl buffer, 1 mM EDTA, pH 7.4. Homogenate was centrifuged 7 min at 700g and, after removal of the pellet, 15 min at 11000g. The sediment was resuspended in a small volume of EDTA-free medium and stored on ice. The protein content was determined by the Lowry method.

Metabolite content was determined in mitochondrial protein-free extractions. For this purpose, aliquots of mitochondrial suspensions were sampled and protein was removed by the addition of 0.5 M $HClO_4$ with consequent precipitation 10 min at 10000g. After the pellet was discarded, supernatant was neutralized by the

additions of 5 M KOH and centrifuged repeatedly for 5 min at 10000g. Protein-free extracts were stored at -70°C and further used for the metabolite analysis. Diene conjugates were determined spectrophotometrically in heptane extracts of mitochondrial preparations [18].

To find the content of LTC_4 and TxB_2 in mitochondria, mitochondrial methanol extracts were concentrated by reverse-phase HPLC on C_{18} columns. The content of metabolites was determined by radioimmunoassay method [19] using radiolabeled standards ("Amersham", UK).

The method for H_2O_2 determination was based on the indirect registration of H_2O_2 consumption in the course of iodide (I^-) oxidation to iodine (I_3^-) in the presence of excess lactoperoxidase, the stoichiometric ratio between H_2O_2 consumption and I_3^- formation being 1:1 [20]. Aliquots of mitochondrial protein-free extractions were incubated in 2 ml of incubation medium which contained 0.1 M KI in the presence of the excess lactoperoxidase (0.5 μg·ml^{-1}) in 0.05M Tris buffer, pH 7.33 (25°C). I_3^- formation was followed spectrophotometrically at 353 nm and the amount of hydroperoxide was determined using molar absorption coefficient 26000 mol^{-1}·cm^{-1}.

Mitochondrial NOS activity was assessed by measuring L-citrulline content in mitochondrial extractions with diacetylmonoxime [21]. To remove urea, which prevented L-citrulline determination with diacetylmonoxime, the probes were pre-incubated with urease [21].

The determination of nitrate was conducted with brucine method based on the reaction of the nitrate with brucine sulfate in a 13 N H_2SO_4 solution at 100ºC. The color complex was measured spectrophotometrically at 410 nm with a nitrate kit ("Felicit diagnostic", Ukraine) [22].

The activity of xanthine oxidase in tissue extracts from heart and liver was assessed based on the level of uric acid, the stable end product of its enzymatic activity. Uric acid was determined spectrophotometrically using uric acid kit ("Felicit diagnostic", Ukraine).

All reagents were from "Merck" (USA). Kits for determination of nitrate, Fe^{2+}, urea, and uric acid were supplied by "Felicit diagnostic" (Ukraine). Radiolabeled probes for the determination of eicosanoids were of "Amersham" (UK). Deionized water was used for solutions preparation.

The data were expressed as mean ± S.E. of 4-6 independent experiments. Correlation dependences and Pearson correlation coefficients R^2 and the reliability of correlations were found using Excel 7.0. Statistical analysis was performed using paired Student's t-test; $P < 0.05$ was taken as the level of significance.

RESULTS

Non-enzymatic lipid oxidation in mitochondria was assessed based on the formation of diene conjugates (Fig. 1A). With the purpose to find the mechanisms regulating lipid oxidation in heart and liver mitochondria, we studied the effect of NG application on hydroperoxide formation, the level of Fe^{2+} in mitochondria, and the activity of xanthine oxidase in heart and liver tissues (Fig. 1C, D). Also, we estimated the effect of NG on mtNOS activity as the main source of enzymatic RNS production in mitochondria, based on the level of L-citrulline and nitrate formation in mitochondria (Fig. 1E, F).

As showed our experiments, NG application caused fast dose-dependent increase in the amount of diene conjugates, which was in line with the increase of hydroperoxide production in heart and liver mitochondria (Fig. 1A, B). Worth noting that hydroperoxide production in liver mitochondria far exceeded the level of H_2O_2 formation found in heart mitochondria (Fig. 1A). Similarly, xanthine oxidase activity in liver exceeded the activity of this enzyme in the heart, which is well agreed with higher level of hydroperoxide, stable product of superoxide transformation, in liver mitochondria (Fig. 1A, C). Free Fe^{2+} content in liver mitochondria too far exceeded the amount of Fe^{2+} found in heart mitochondria (Fig. 1D). However, in spite of

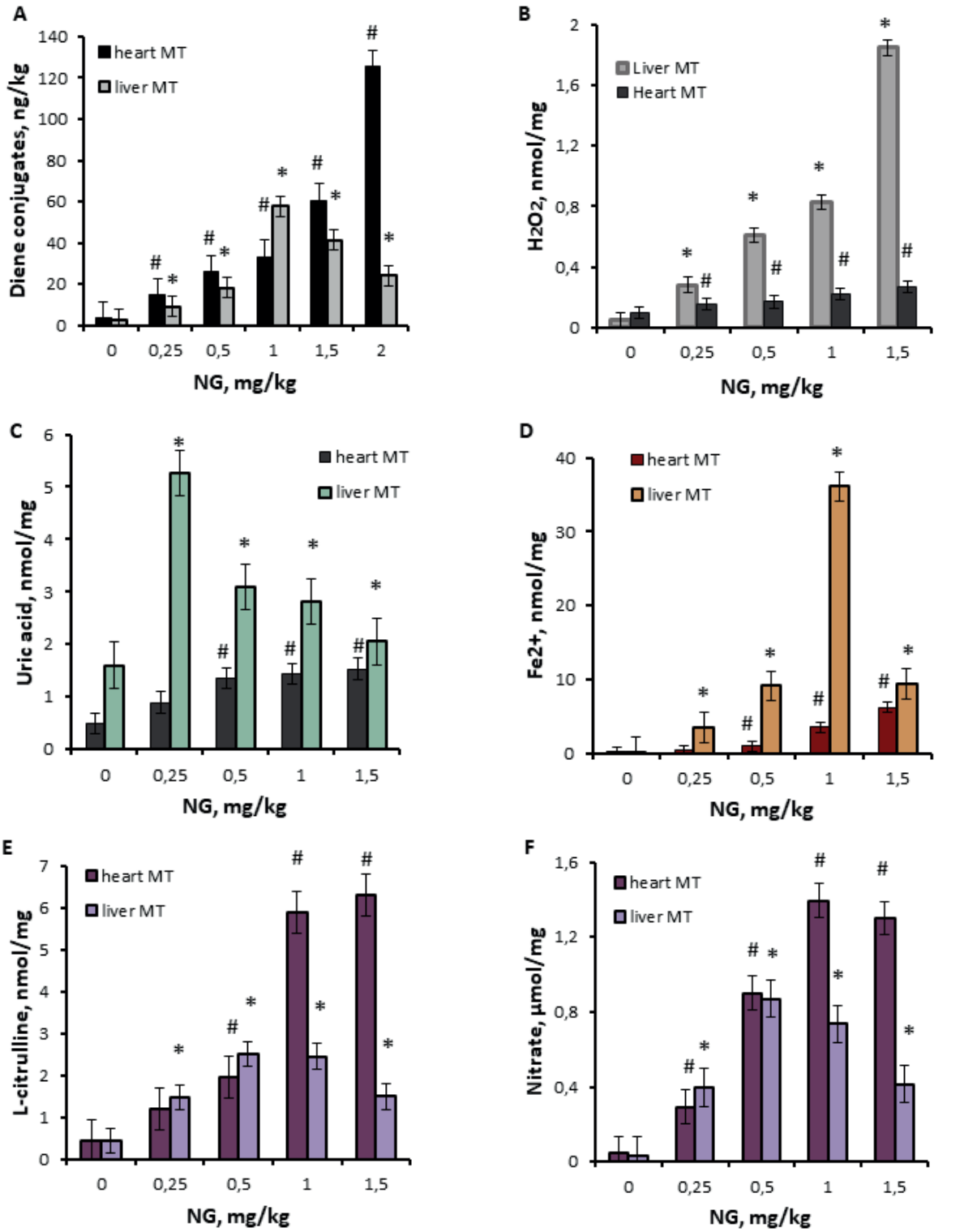

Fig. 1. The effect of NG application on hydroperoxide production (A), DC formation (B), xanthine oxidase activity (C), Fe^{2+} release (D), L-citrulline and nitrate formation ((E, F). M ± m, n = 6. *P < 0.05 as compared to control; #P < 0.05 as compared to liver mitochondria

much higher level of prooxidant factors in liver mitochondria, the levels of DC in heart mitochondria exceeded one found in liver mitochondria, especially at high doses of NG (Fig. 1A).

As we have shown recently [9], there was different regulation of ROS and RNS production in heart and liver mitochondria under NG application. So, fast increment of DC formation in heart mitochondria, as compared to liver ones, indicated different regulatory mechanisms of lipid oxidation as well. All the above allowed us suppose that DC formation in heart mitochondria could be affected by combined action of prooxidants, highly favorable for lipid oxidation in these organelles. So, we made an attempt to disclose underlying mechanisms of these phenomena by finding the correlation dependences between prooxidant factors and DC production in mitochondria. For this purpose, we plotted the amount of DC against the levels of prooxidants found in mitochondria under NG application and estimated Pearson correlation coefficients and the reliability of correlations.

As we observed, there were linear dependences of the level of DC formation on hydroperoxide production in both heart and liver mitochondria, and strong reliable correlation between DC formation and the level of H_2O_2 was found (Fig. 2A). Also, non-enzymatic lipid peroxidation was strongly dependent on free

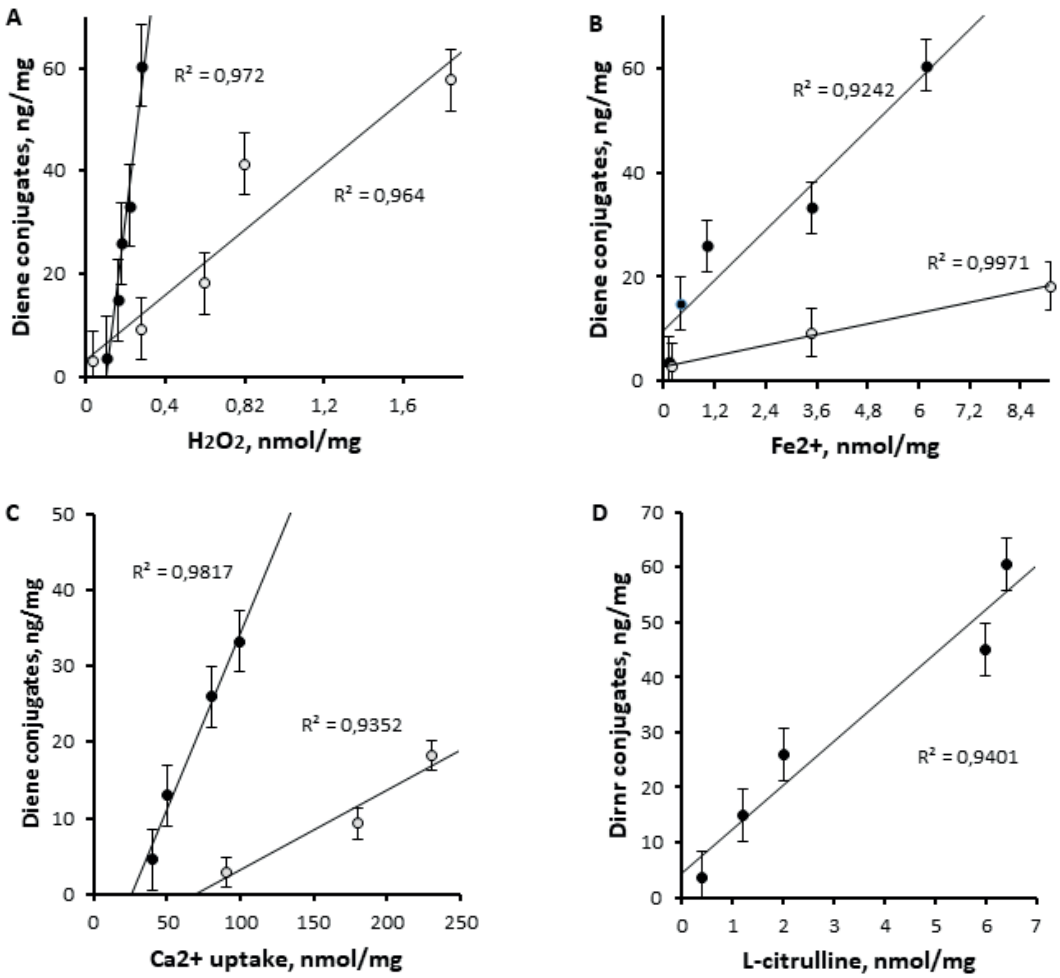

Fig. 2. The correlation dependences between DC formation and H_2O_2 production (A), Fe^{2+} release (B), Ca^{2+} uptake (C), and mtNOS activity (D) in heart (black circles) and liver (grey circles) mitochondria. M ± m, n = 6. *P < 0.05

Fe^{2+} release in both heart and liver mitochondria (Fig. 2B). This indicated that DC formation in mitochondria with high probability could result from the combined action of H_2O_2 and free Fe^{2+} and possible involvement of $^{\cdot}OH$-radical in lipid oxidation [23].

As we have shown earlier, NG administration led to the elevation of Ca^{2+} uptake in mitochondria caused by the blockage of mPTP [5]. As we have found, there was a correlation between Ca^{2+} uptake and the activation of mtNOS and RNS production [5]. It is well known too that Ca^{2+} uptake in mitochondria could trigger multiple mechanisms of ROS production including the respiratory chain and several Ca^{2+} dependent oxidases [24, 25]. So, Ca^{2+} uptake could promote ROS formation indirectly, by the activation of Ca^{2+} dependent enzymes. In agreement with this knowledge, in both heart and liver mitochondria, we observed strong dependence of lipid oxidation on Ca^{2+} uptake (Fig. 2C).

Of other factors affecting DC production, strong highly reliable correlation between DC formation and mtNOS activity was found in heart, but not liver mitochondria (Fig. 2D). Also, DC formation in both heart and liver mitochondria was independent of nitrosothiols (not shown). This indicated that DC formation was dependent on RNS produced enzymatically by mtNOS, but independent of direct nitrosylation of mitochondrial proteins caused by NG application.

As for enzymatically produced eicosanoid species, leukotriene and thromboxane, the dose-dependent increase of its production as well was observed in heart and liver mitochondria (Fig. 3A, B). Both $TxB_2$and LTC_4 formation was highly dependent on H_2O_2 production (Fig. 3C, D; Fig. 4). The formation of these species in heart and liver mitochondria too was strongly dependent on free Fe^{2+} content (Fig. 3E; Fig. 4). Similar to DC production, enzymatic lipid oxidation in heart mitochondria was reliably dependent on mtNOS activity in heart mitochondria (Figs. 3F; 4), but no such dependence was found in liver mitochondria. Also, LTC_4 and TxB_2 production in heart and liver mitochondria was independent on nitrosothiols formation (Fig. 4). Both enzymatic and non-enzymatic lipid oxidation in heart and liver mitochondria was independent of xanthine oxidase activity (not shown).

It was remarkable that in heart mitochondria there was much steeper rise in all lipid oxidation products, DC, LTC_4, and TxB_2, as compared to liver ones (Fig. 2; 3). This indicated different regulatory mechanisms of lipid oxidation in heart and liver mitochondria and combined effects of different prooxidant factors on lipid oxidative metabolism.

DISCUSSION

Mitochondria possess multiple sources of ROS produced by the respiratory chain and matrix prooxidants [24, 25]. Xanthine oxidase, an enzyme producing superoxide, is a potent source of cellular ROS, and is considered as one of the main sources of superoxide implicated in the development of several pathologies and cardiovascular diseases [26]. Another potent prooxidant factor is free Fe^{2+} that could be released from iron-containing proteins abundant in mitochondria. Cytotoxic potential of Fe^{2+} is based on its ability to react with H_2O_2 by the mechanisms of Fenton and Haber-Weiss reactions with the formation of highly toxic $^{\cdot}OH$ radical, which in turn could react with membrane lipids initiating chain reactions with the formation of lipid radicals: $Fe^{2+} + H_2O_2 \rightarrow \cdot OH + OH^- + Fe^{3+}$ [23].

However, based on our experiments, no correlation existed between lipid oxidation and xanthine oxidase activity, which allowed us suppose minor role of cytosolic superoxide produced by xanthine oxidase in lipid oxidation mechanisms in mitochondria under NG application. Instead, there was strong highly reliable correlation between H_2O_2 production in mitochondria, free Fe^{2+} release and lipid oxidation products (Fig. 4), which indicated high probability of Fe^{2+} involvement in oxidation processes *via* interaction with H_2O_2 and the formation of $^{\cdot}OH$ radical.

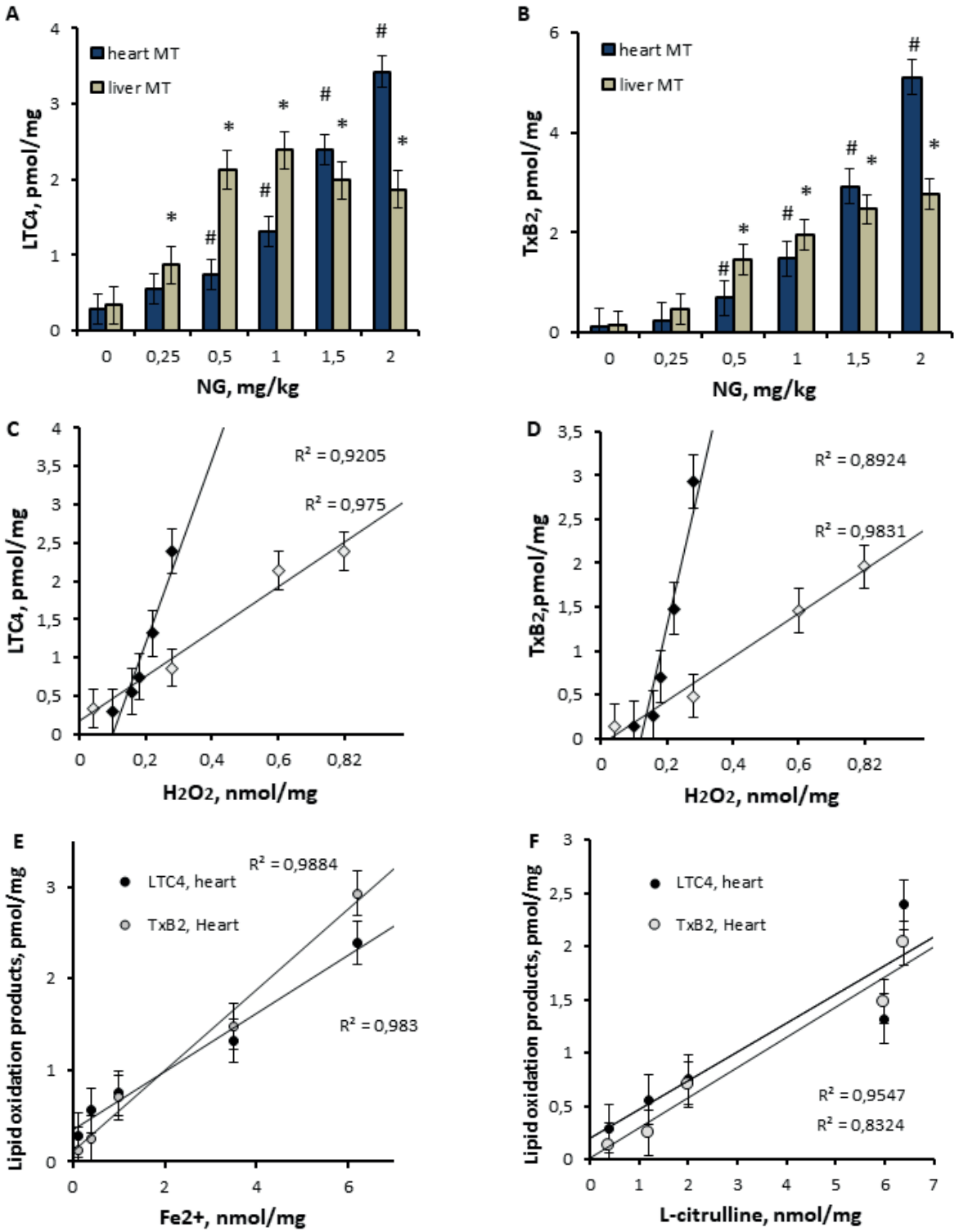

Fig. 3. LTC_4 and TxB_2 formation in heart and liver mitochondria (A, B) and the dependences of LTC_4 and TxB_2 formation on H_2O_2 production in heart and liver mitochondria (C, D, black and grey circles), Fe^{2+} release and mtNOS activity in heart mitochondria (E, F). M ± m, n = 6. *P < 0.05

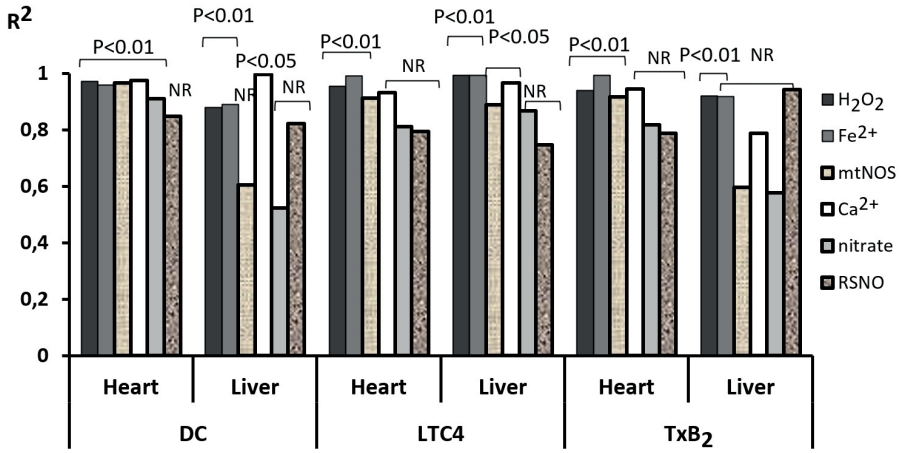

Fig. 4. Pearson coefficients (R^2) of the correlation dependences between lipid peroxidation products and factors regulating ROS and RNS production in mitochondria (hydroperoxide formation, Fe^{2+} release, Ca^{2+} uptake, mtNOS activity, nitrate production, and non-enzymatic protein nitrosylation). n = 6. The results of statistical analysis are shown on the plot; NR – not reliable

Ca^{2+} uptake too could promote ROS and RNS formation by acting on the respiratory chain, mtNOS, and several Ca^{2+}-dependent mitochondrial oxidases capable of producing superoxide [24, 25, 27]. So, an increase in Ca^{2+} uptake caused by the blockage of mPTP [5] could be favorable for lipid oxidation in mitochondria. In agreement with this notion, we observed reliable dependence of DC formation on Ca^{2+} uptake in heart and liver mitochondria (Fig. 2C; Fig. 4).

Rather unexpected was a strong highly reliable correlation between DC formation and mtNOS activity in heart, but not liver, mitochondria, which could indicate the dependence of non-enzymatic lipid oxidation in heart mi-

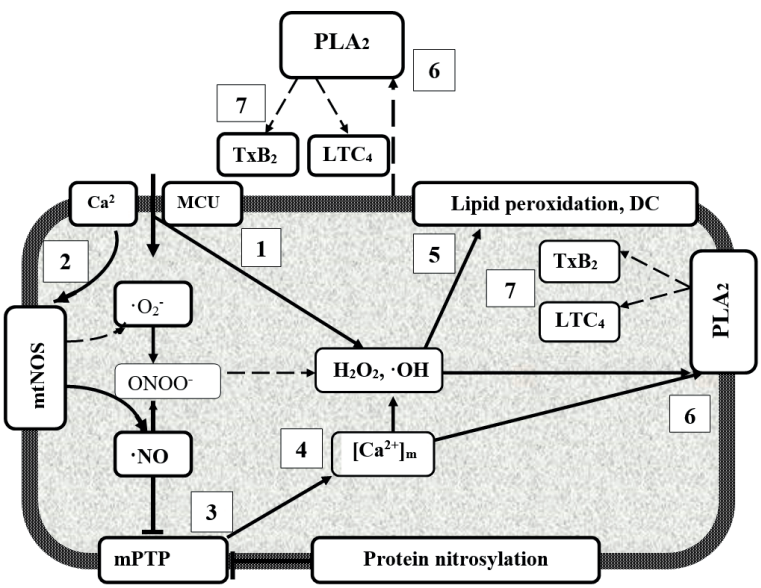

Fig. 5. A scheme showing putative steps of lipid peroxidation under NG application. 1 – Ca^{2+} uptake *via* MCU and the increase of H_2O_2 production. 2 – activation of mtNOS and NO formation. Possible formation of superoxide, peroxynitrite, and·OH radical. 3 – MPTP blockage by NO and direct non-enzymatic nitrosylation. 4 – increase in matrix Ca^{2+} as a result of mPTP blockage. 5 – non-enzymatic lipid peroxidation by mitochondrial ROS. 6 – activation of mitochondrial PLA_2 by Ca^{2+} and ROS; activation of cytosolic PLA_2 by mitochondrial H_2O_2. 7 – putative pathways of LTC_4 and TxB_2 formation following PLA_2 activation

tochondria on mtNOS activity. To explain observed phenomenon, we need to consider much higher mtNOS activity in heart mitochondria and suppression of mtNOS activity in liver mitochondria at high doses of NG. It worth mention, that no dependence of lipid oxidation on nitrosothiols formation was found. This observation was well in line with the notion that lipid oxidation could be promoted by $^{\cdot}NO$ and $^{\cdot}NO_2$ radicals, which are the products of enzymatic NOS activity. As it was shown in the literature, both $^{\cdot}NO$ and $^{\cdot}NO_2$, but not nitrosothiols, could react with superoxide with the formation of peroxynitrite, which decomposition could produce $^{\cdot}OH$ radical. This mechanism is capable of strong lipid oxidation, even without involvement of Fe^{2+} [10, 28]: $NO + {\cdot}O_2^- \rightarrow ONOO^- + H^+$(1); $ONOO^- + H^+ \rightarrow ONOOH$ (2); $ONOOH \rightarrow {\cdot}NO_2 + OH$ (3). Considering that NOS in the case of its uncoupling could produce both ·NO and superoxide, it is tempting to hypothesize that mtNOS, highly activated by Ca^{2+} uptake in heart mitochondria, could be a source of peroxynitrite and ·OH radical. This assumption agrees with literary data, which showed peroxynitrite production by mtNOS activated by mitochondrial Ca^{2+} uptake [27].

The above considerations could explain the observed differences in the regulation of lipid oxidation in heart and liver mitochondria. MtNOS activity was suppressed in liver mitochondria, especially at high doses of NG (Fig. 1E, F), so its participation in lipid oxidation in liver mitochondria was not likely (Fig. 4). On the contrary, high mtNOS activity in the heart could largely contribute to lipid oxidation and explain much steeper rise in DC formation in heart than in liver mitochondria (Figs. 2; 3). The same putative mechanism could explain elevated production of thromboxane and leukotriene and faster increment in the production of these species in heart as compared to liver mitochondria (Fig. 3C, D).

Thromboxanes and leukotrienes are commonly known markers of oxidative stress and inflammation [12]. So, observed increase in the production of these species was in line with the elevation of prooxidant production in mitochondria, especially at high doses of NG. To explain the effect of NG application on thromboxane and leukotriene production, one needs to consider that these species are downstream products of PLA_2/arachidonic acid cascade, which is activated by ROS and Ca^{2+} [11, 12]. So, increase in ROS production facilitated by the involvement of free Fe^{2+} could result in the activation of mitochondrial PLA_2 and promote the formation of its downstream products. This could be a plausible explanation for the observed increase in LTC_4 and TxB_2 formation, which showed strong reliable dependence on hydroperoxide production and Fe^{2+} release in heart and liver mitochondria (Fig. 4). Also, PLA_2 could be directly activated by the elevation of matrix Ca^{2+}[13] however, in this work we failed to establish a correlation between Ca^{2+} uptake and the formation of lipid metabolites of PLA_2 cascade.

Similar to non-enzymatic lipid oxidation, there was a strong reliable correlation between LTC_4 and TxB_2 formation, and mtNOS acxtivity in heart mitochondria, which indicated possible dependence of eicosanoid production on NOS-produced RNS. As in the case of DC formation, we assumed that high output of RNS in heart mitochondria, capable to produce peroxynitrite and $^{\cdot}OH$ radical, could contribute to the activation of PLA_2 and consequently promote formation of LTC_4 and TxB_2 in heart mitochondria. This scenario was not likely to occur in liver mitochondria, which mtNOS activity was twice as lower as in heart mitochondria, and was suppressed under high doses of NG (Fig. 1E, F). These considerations were confirmed by the absence of correlations between lipid oxidation and mtNOS activity in liver mitochondria (Fig. 4).

One unresolved issue in our work is the source of thromboxane B_2 and leukotriene C_4 found in mitochondrial extracts under the action of NG. Over the past decade a progress was reached in the understanding of the mechanisms of eicosanoids formation and their physiological functions. However, the mechanisms of their

formation in mitochondria are little studied yet. Generally, it is known that leukotrienes and thromboxanes are formed outside mitochondria upon the activation of cytosolic PLA_2 by ROS and other stimuli [11, 12]. However, it was shown that these species can be transported to mitochondria and take part in β-oxidation [17], which deeply affects mitochondrial functions and bioenergetics.

Mitochondria possess several isoforms of phospholipase A_2, which are activated by ROS, particularly H_2O_2, but biological functions of mitochondrial phospholipases A_2, especially Ca^{2+}-independent $iPLA_2\gamma$ implicated in producing bioactive lipid mediators [13, 16], over decades remained unclear. However, in the past decade evidence was obtained of the formation of arachidonic acid mediated by the hydrolysis of oxidized cardiolipin by Ca^{2+}-independent PLA_2 in mitochondria [15]. Also, the activation of iPLA2γ by Ca^{2+} and Mg^{2+} was shown in heart mitochondria with consequent Ca^{2+} stimulated production of arachidonic acid from endogenous mitochondrial phospholipids and accumulation of the downstream products of their enzymatic oxidation by lipoxygenases and cyclooxygenases, particularly multiple HETE homologs, TxB_2 and LTB_4 [13].

Considering the above literary data, the formation of eicosanoids in mitochondria under the action of NG cannot be ruled out. Although at present we have no precise knowledge about the mechanism and the site(s) of the formation of LTC_4 and TxB_2 under the action of NG *in vivo*, the above literary findings allow us hypothesize that these lipid mediators found in mitochondrial extractions, at least partially, could be of mitochondrial origin. Proposed regulatory mechanisms of lipid oxidation in mitochondria are summarized by a scheme (Fig. 5). Based on our experiments, in this work we reached following conclusions:

CONCLUSIONS

1. NG application resulted in dose-dependent formation of lipid oxidation products: diene conjugates (DC), leukotriene C_4 (LTC_4), and thromboxane B_2 (TxB_2) in heart and liver mitochondria. Formation of DC, LTC_4, and TxB_2 was strongly dependent on hydroperoxide production and free iron release in mitochondria. DC formation exhibited the dependence on Ca^{2+} uptake. No dependence of lipid oxidation on xanthine oxidase activity, or nitrosothiols formation was found.

2. As showed correlation dependences, in heart mitochondria DC, LTC_4, and TxB_2 formation exhibited strong dependence on mtNOS activity. No dependence of lipid oxidation on mtNOS activity in liver was found. This was in line with much higher mtNOS activity in heart mitochondria, and suppression of mtNOS activity in liver mitochondria at high doses of NG. This is agreed with the observation that lipid oxidation was independent of direct protein nitrosylation caused by NG application.

3. Hydroperoxide production, free Fe^{2+} release, and lipid peroxidation were regulated differently in heart and liver mitochondria. While the level of prooxidants was much higher in liver mitochondria, much steeper rise in lipid oxidation products was observed in heart mitochondria. To find a plausible explanation for observed phenomena, we hypothesized that RNS overproduction caused by the elevated mtNOS activity in the heart could largely contribute to lipid peroxidation and promote much faster increase in the formation of lipid oxidation products in heart as compared to liver mitochondria.

4. The mechanisms regulating lipid peroxidation in mitochondria caused by the application of NO donors, partially NG, need extensive further studies. Obtained correlation dependences allowed us disclose principal factors affecting lipid peroxidation in mitochondria under NG administration *in vivo*.

The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations

and/or individuals who may have been related to the study, and interrelations of co-authors of the article.

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РЕГУЛЯЦІЯ ПЕРЕКИСНОГО ОКИСНЕННЯ ЛІПІДІВ У МІТОХОНДРІЯХ ЗА ДІЇ НІТРОГЛІЦЕРИНУ

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Досліджено механізми окиснення ліпідів в ізольованих мітохондріях серця та печінки щурів за дії нітрогліцерину (НГ). Показано дозозалежне утворення дієнових кон'югатів (ДК), лейкотрієну С4 (LTC_4) і тромбоксану B_2 (TxB_2) за дії НГ. Для виявлення механізмів, що регулюють пероксидацію ліпідів у мітохондріях, вивчали вплив НГ на утворення прооксидантів (H_2O_2 та вільного заліза Fe^{2+}) а також активність ксантиноксидази та мітохондріальної (mtNOS) як основних джерел активних форм кисню і азоту (АФК і АФА відповідно). На підставі кореляційних залежностей встановили строгу кореляційну залежність утворення ДК, LTC_4, і TxB_2 від генерації гідропероксиду та вивільнення заліза в мітохондріях. Утворення ДК також залежало від акумуляції Ca^{2+} в мітохондріях. Окиснення ліпідів не залежало від активності ксантиноксидази. Проте в мітохондріях серця виявлено строгу залежність утворення ДК, LTC_4, та TxB_2 від активності mtNOS. При цьому окиснення ліпідів не залежало від нітрозилювання білків та утворення нітрозотіолів внаслідок дії НГ. У мітохондріях печінки воно не залежало від активності mtNOS, що узгоджується з набагато більшою її активністю в мітохондріях серця та пригніченням в мітохондріях печінки із збільшенням дози НГ. Дійшли висновку, що за дії НГ гіперпродукція АФК та вивільнення Fe^{2+} сприяли ензиматичному та неензиматичному окисненню ліпідів в мітохондріях серця і печінки. Також ми припускаємо, що гіперпродукція АФА внаслідок високої активності mtNOS в мітохондріях серця сприяла прискореному утворенню продуктів окиснення ліпідів порівняно з мітохондріями печінки, особливо за високих доз НГ. Отже, на підставі одержаних кореляційних залежностей, виявлено, що вільне залізо, гідропероксид та активність mtNOS є основними чинниками регуляції окиснення ліпідів у мітохондріях за дії НГ.

Ключові слова: нітрогліцерин; серце; печінка; мітохондрії; АФК; АФА; пероксидація ліпідів; mtNOS.

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