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The regulation of mitochondrial NO synthase activity under nitroglycerine application in rat heart and liver mitochondria

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Nitroglycerine (NG) affords cardioprotection via NO formation, but the impact of NG application on reactive nitrogen species (RNS) metabolism remains little studied yet. Mitochondrial NO synthase (mtNOS) is an important endogenous source of RNS. Our aim was to study the effect of NG application on mtNOS activity and RNS production in rat heart and liver mitochondria. Different regulation of mtNOS activity in the heart and liver under NG treatment was found. While in heart mitochondria it increased dose-dependently, in liver mitochondria only moderate elevation and bell-shaped dose dependence of mtNOS activity on NG was observed. Nitrite and nitrate, which are the end products of L-arginine transformation by NOS, showed similar dose dependence on NG. To find an explanation for the observed dependences, we studied the effects of NG administration on the activity of arginase which competes with NOS for physiological substrate, L-arginine. A strong reciprocal dependence between mtNOS and arginase activities was found. As we observed, the arginase activity increased under NG application. However, while in heart mitochondria high mtNOS activity agreed with moderate arginase activation, in liver mitochondria high arginase activity coincided with suppression of mtNOS activity at high doses of NG. Low arginase and high mtNOS activities observed in heart mitochondria were consistent with high NO_2^- and NO_3^- production and low hydroperoxide (H_2O_2) formation, whereas high arginase activity in liver mitochondria was accompanied by the reduction of NO_2^-/NO_3^- formation and simultaneous elevation of H_2O_2 production. A linear correlation between the arginase activity and hydroperoxide formation was found. We came to the conclusion that under NG administration arginase activation resulted in reciprocal regulation of RNS and ROS production in mitochondria, dependent on the proportion of mtNOS to arginase activity. Suppression of RNS metabolism could be the cause of ROS overproduction caused by high arginase and low mtNOS activity.

Key words: heart; liver; mitochondria; NOS; arginase; reactive nitrogen and oxygen species.

INTRODUCTION

Nitroglycerine (NG) is known as a cardioprotective drug for over 100 years [1], but molecular mechanisms underlying its the cardioprotective action are not quite clear. It is generally assumed that protective action of NG is afforded by the formation of nitric oxide (NO), which triggers a complex network of signaling pathways, primarily cGMP/PKG signaling, resulting in cardioprotection [2]. Both NO deficiency and NO hyperproduction under pathophysiological conditions result in dramatic dysregulation of RNS and ROS production and the impairment of NO-dependent regulation of vessel tone and heart function [2-4].

NO, which is primarily a product of enzymatic activity of constitutive and inducible NO synthases, undergoes a series of redox modifications. The main steps of NO transformations include protein nitrosylation and S-nitrosothiols formation, NO oxidation to nitrite and nitrate, and NO recovering by nitrate and nitrite reductases, which on a whole constitutes so-called NO cycle [5]. Mitochondria comprise both primary (mtNOS) and the end steps of NO cycle (nitrate reductase), which makes these organelles important players of cellular RNS metabolism.

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In the cellular milieu, NO is rapidly delivered from NG, but the impact of NG as NO donor on RNS production remains little studied yet. Partially, the cardioprotective effects of NG could be explained by direct protein nitrosylation [2]. Earlier we have shown increased mitochondrial protein nitrosylation under NG application accompanied by the inhibition of permeability transition pore [6]. Recently, it was shown direct S-nitrosylation of cyclophilin D, one of the main components of the pore, caused by NG application [7] However, there is still scarce information about RNS metabolism in mitochondria under NG treatment. As it is known, mitochondria possess a Ca^{2+} dependent constitutive isoform of NO synthase (mtNOS), which is close to neuronal NOS by its molecular composition [8]. MtNOS is ubiquitously present in all cell types. Being one of the main endogenous sources of RNS in mitochondria, mtNOS activity is one of the key regulators of mitochondrial functions. Thus, NO production in mitochondria was shown to control respiration via nitrosylation and inhibition of respiratory chain complexes I, III and IV with ensuing modulation of ROS formation [9-11]. NO-dependent inhibition of mitochondrial respiration is thought to be protective under ischemic and hypoxic conditions [10]. However, regardless of the importance of NO for mitochondrial functions, the studies on RNS metabolism in mitochondria still are at their beginning. Also, for a better understanding of the impact of NG treatment on mitochondrial functions, it is important to evaluate the effect of this drug on NO transformations in mitochondria and assess their possible cell specificity.

One important factor limiting NOS activity is the activity of arginase, an enzyme, which competes with NOS for the same substrate, L-arginine [11-13]. Arginase hydrolyses L-arginine to ornithine and urea [13]. Similar to NOS, arginase is ubiquitously expressed in the vertebrates' tissues. Both cytosolic (arginase I) and mitochondrial (arginase II) isoforms are known [14]. Arginase exhibits a much lower affinity for L-arginine, (K_m ~ 1-5 mM), as compared to NOS (K_m ~ 2-20 μM), but 1000 times higher V_{max} [12, 13]. Up-regulation of arginase may reduce the availability of L-arginine for mtNOS activity, which eventually may be a cause of NOS uncoupling. Under numerous pathophysiological conditions, reciprocal regulation of NOS and arginase activities was observed, i.e. up-regulation of arginase and down-regulation of NOS activity [15-17] and *vice versa*, inhibition of arginase by the high NOS activity at the high rate of NO production too was shown [18].

As we have shown earlier, mtNOS activity was increased in heart and liver mitochondria under NG treatment *in vivo*, caused by the elevation of Ca^{2+} uptake [6]. This occurred in parallel with strong activation of RNS and ROS production in these organelles. So, for a better understanding of the mechanisms regulating RNS production in mitochondria under the action of NG, it was of interest to evaluate the interplay between mtNOS and mitochondrial arginase activities under NG application. For the purpose to examine further possible cell-specific effects of NG treatment at the level of mitochondria, the aim of this work was to compare the effects of NG application on mtNOS and arginase activities, as well as RNS and ROS production in heart and liver mitochondria.

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 Ukraine. Adult Wistar rats with 180-200 g body weight were used. Nitroglycerine (NG) was administered intraperitoneally at doses 0.25, 0.5, 1.0 and 1.5 mg/kg weight. Control animals were administered a 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 for 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.

RNS production was assessed by the determination of stable metabolites content: nitrite, nitrate, and S-nitrosothiols. Mitochondrial NOS activity was evaluated by measuring L-citrulline content in mitochondrial extractions. For the determination of metabolite content in mitochondrial preparations 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 used for the metabolite analysis.

Nitrite content was determined by the method of Green with the Griess reagent [19]. Griess reagent was prepared by mixing equal volumes of 0.1% water solution of N-(1-naphthyl)ethylenediamine dihydrochloride with 1% sulfanilamide in 5% H_3PO_4 just before use. 0.5 ml aliquots of mitochondrial protein-free extractions were sampled and mixed with Griess reagent in a proportion of 1:1 by volume. 5 min after absorbance at 546 nm was measured; nitrite concentration was determined from calibration curves.

The determination of nitrate was conducted with the brucine method based upon the reaction of the nitrate with brucine sulfate in a 13 N H_2SO_4 solution at 100°C. The color complex was measured spectrophotometrically at 410 nm with a nitrate kit ("Felicit diagnostic", Ukraine) [20].

Total S-nitrosothiol content was determined by the method of Saville [21], using Griess reagent with $HgCl_2$ for S-nitrosothiol decomposition. Briefly, $HgCl_2$/sulfanilamide solution was prepared by mixing 1 volume of 1% $HgCl_2$ in water with 4 volumes of 3.4% sulfanilamide in 0.4 M HCl. 0.5 ml aliquots of mitochondrial suspensions, were added to 0.7 ml $HgCl_2$/sulfanilamide solution and 5 min after mixed with 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride in 0.4 M HCl in a proportion 2:1 by volume. Control probes were treated as above but without $HgCl_2$. After protein removal by centrifugation and the neutralization of the probes, absorbance at 546 nm was measured. S-nitrosothiol content was determined from the increment in nitrite concentration vs. controls using calibration curves.

Mitochondrial NOS activity was assessed by measuring L-citrulline content in mitochondrial extractions with diacetyl monoxime [18]. To remove urea, which prevented L-citrulline determination with diacetyl monoxime, the probes were pre-incubated with urease [18].

Arginase activity was assessed based on the determination of urea, which is the end product of arginase reaction, with diacetyl monoxime using the urea kit ("Felicit diagnostic", Ukraine). The activity was expressed as the amount of urea, $nmol \cdot min^{-1} \cdot mg^{-1}$ protein.

NADH-dependent nitrate reductase activity was found based on the determination of nitrate as above. The activity was expressed as the amount of reduced nitrate, $nmol \cdot min^{-1} \cdot mg^{-1}$ protein [20].

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, stoichiometric ratio between H_2O_2 consumption and I_3^- formation being 1:1 [22]. 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 $\mu g \cdot ml^{-1}$) in 0.05M Tris buffer, pH 7.33 (25°C). I3$^-$ formation was followed spectrophotometrically at 353 nm and the amount of hydroperoxide was determined using molar absorption coefficient 26000 $mol^{-1} \cdot cm^{-1}$.

All reagents were from "Merck" (USA). Kits for the determination of nitrate and urea were supplied by "Felicit diagnostic" (Ukraine). Deionized water was used for solutions preparation.

The data were expressed as mean ± s.e. of 4-6 independent experiments. Statistical analysis was performed using paired Student's t-test; $P < 0.05$ was taken as the level of significance.

RESULTS

As we observed, NG administration resulted in different regulation of mtNOS activity in heart and liver mitochondria (Fig. 1A). The changes in mtNOS activity in vivo were assessed based on the determination of its product, L-citrulline. In heart mitochondria, mtNOS activity increased upon application of up to 1 mg/kg NG, which was in parallel with the increase of nitrite and nitrate formation (Fig. 1) and agreed with the dependence of mtNOS activity on mitochondrial Ca^{2+} uptake established in our previous work [6].

However, while basal mtNOS activity was quite similar in heart and liver mitochondria, different dependence of mtNOS activity on NG doses was found (Fig. 1A). Unlike heart mitochondria, in liver mtNOS activity was much more suppressed and showed a bell-shaped dependence resulting in the decrease by about a half of its maximal level at high doses of the drug (Fig. 1A). Nitrate (NO_3^-) and nitrite (NO_2^-) contents showed similar dose-dependence. At NG doses above ~0.5 mg/kg both nitrate and nitrite production in liver mitochondria was dose-dependently suppressed, which was in line with similar changes in mtNOS activity (Fig. 1A-C). Consequently, starting from ~1.0 mg/kg NG, suppression instead of activation of both mtNOS and NO_2^-/NO_3^- formation, was found in liver mitochondria.

Unlike NO_2^-/NO_3^- production, S-nitrosothiols content did not seem to correlate with the changes in mtNOS activity and showed a plain stepwise increase in parallel with the increase of the dose of NG (Fig. 1D). Also, we did not observe essential differences in S-nitrosothiol formation in the liver as compared to heart mitochondria.

It is well known that mtNOS activity and RNS metabolism constitute the so-called "NO cycle" [5]. MtNOS activity and the total yield of RNS are dependent on concurrent interactions with other enzymes capable of consuming either substrate (L-arginine) or nitrate (the end product of the NO cycle). Under *in vivo* conditions, NOS is known to compete with arginase for the same substrate, L-arginine. Thus for the purpose to explain observed discrepancy in the regulation of mtNOS activity and RNS production, we studied the effect of NG application on the activity of nitrate reductase known to reduce nitrate to nitrite and the activity of arginase known to compete with NOS for L-arginine.

As shown in the experiments, nitrate reductase activity exhibited similar bell-shaped dependence on NG as mtNOS. Peak activation of nitrate reductase was observed at 0.5 (liver) and 1.0 mg/kg NG (heart) followed by the suppression of the enzyme activity (Fig. 2B). In liver mitochondria, above 0.5 mg/kg NG nitrate reductase activity was strongly dose-dependently suppressed, while in heart mitochondria the inhibition of nitrate reductase activity was observed only at high doses of NG, above 1 mg/kg (Fig. 2A). The observed decrease in nitrate reductase activity, most noticeable in liver mitochondria, was in line with the reduced availability of nitrate, which well agreed with the decrease in mtNOS activity. Thus the reduction of nitrate formation under NG treatment correlated with the reduction of mtNOS activity, and could not be explained by the activation of nitrate reductase.

While basal levels of both mtNOS and arginase activities, as well as NO_3^- production were quite similar in heart and liver MT, the experiments showed different dose-dependent regulations of arginase activity. Thus, while NG application in both cases increased the enzyme activity, only a moderate increment of arginase activity was observed in heart mitochondria, whereas steep activation of the

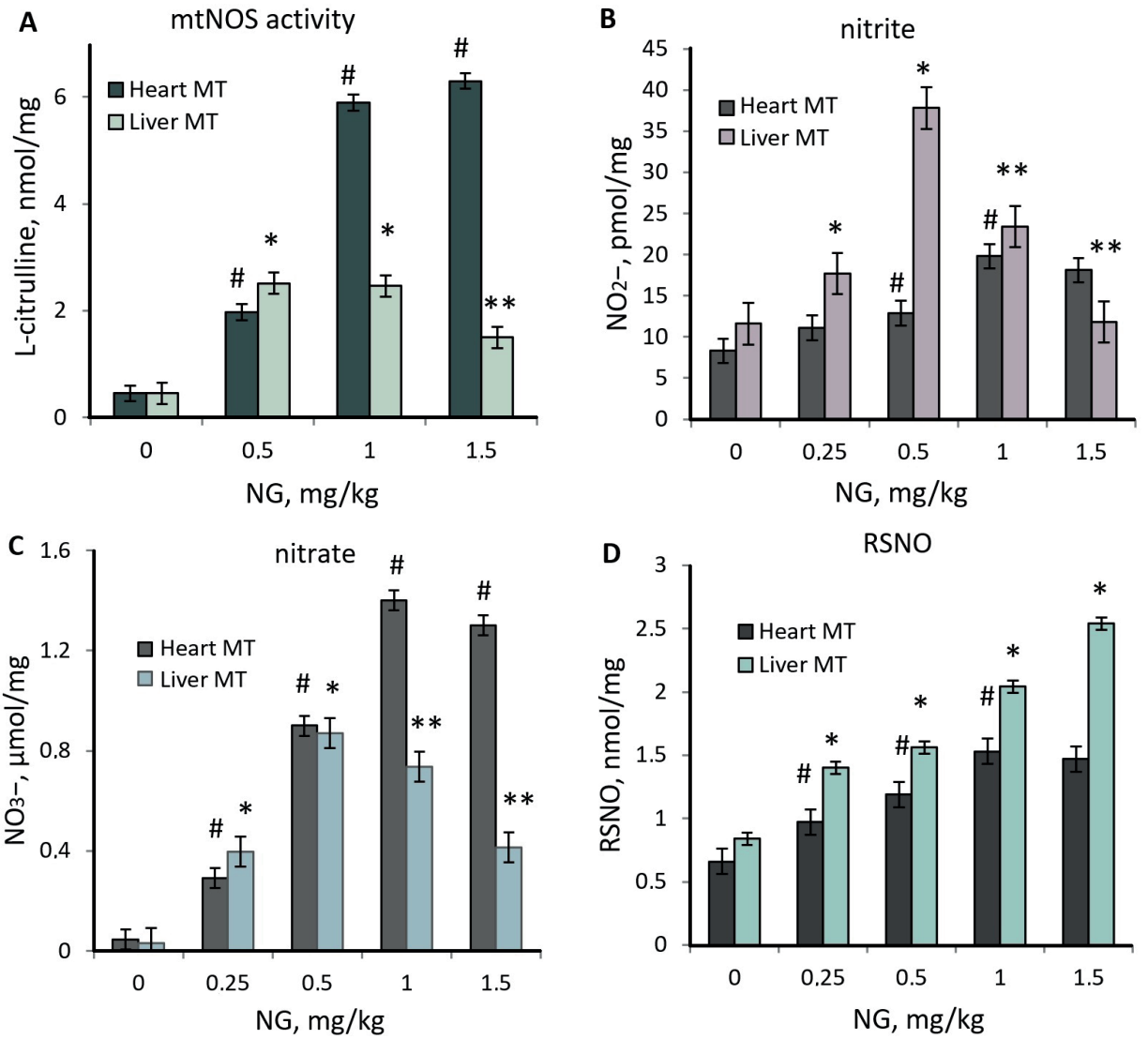

Fig. 1. L-citrulline (A), nitrite (B), nitrate (C), and nitrosothiols formation (D) under NG administration in heart and liver mitochondria. M ± m, n = 4; #P < 0.05 as compared to control (heart); *P < 0.05 as compared to control (liver); **P < 0.05 as compared to 0.5 mg/kg NG

enzyme was observed in liver mitochondria (Fig. 2B). Obtained results showed that high mtNOS activity in heart mitochondria coincided with moderate arginase activation, whereas high arginase activity in liver mitochondria coincided with apparent suppression of mtNOS activity, which indicated a reciprocal regulation of mtNOS and arginase activity. Accordingly, low arginase activity in heart mitochondria correlated with high citrulline and NO_2^-/NO_3^- production, and *vice versa*, high arginase activity in liver mitochondria was accompanied by much lower citrulline and NO_2^-/NO_3^- content (Figs. 1; 2). Moreover, high arginase activity at high doses of NG corresponded to dose-dependent suppression of both mtNOS activity and NO_2^-/NO_3^- formation in liver mitochondria (Fig. 1A-C).

So, assuming a reciprocal regulation of mtNOS and arginase activities observed in our experiments, we have tried to build up dependences of citrulline, nitrite, and nitrate

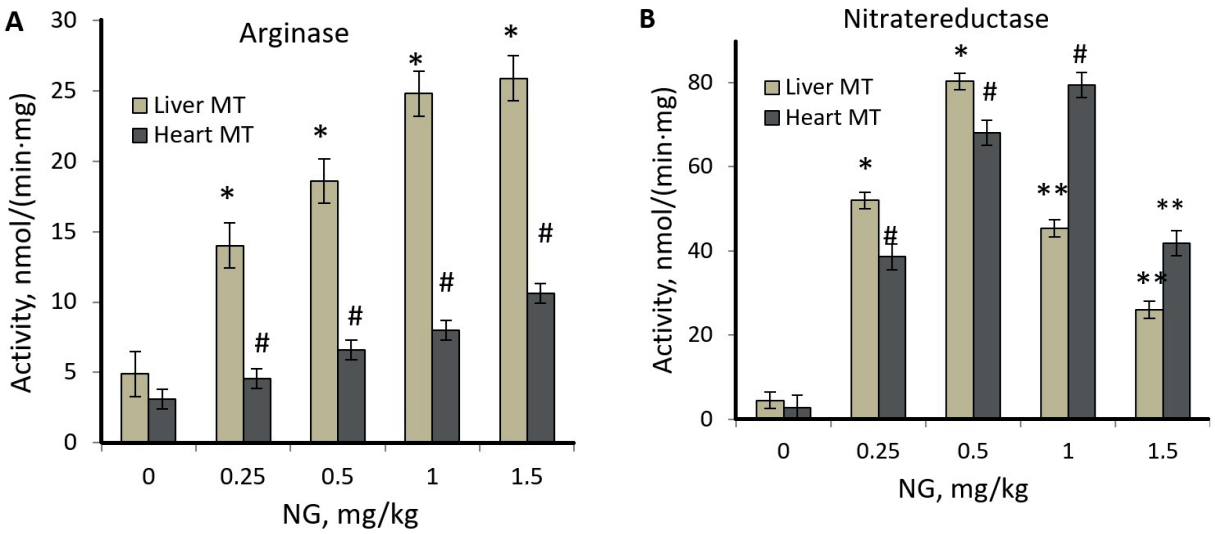

Fig. 2. The effect of NG application on arginase and nitrate reductase activity in rat heart and liver mitochondria. M ± m, n = 4; #P < 0.05 as compared to control (heart); *P < 0.05 as compared to control (liver); **P < 0.05 as compared to 0.5 mg/kg NG

formation, which all correlated with mtNOS activity, on arginase activity found in heart and liver mitochondria. For this purpose, all obtained data on arginase activity were put together and plotted on the abscissa axis in order of increasing values. L-citrulline and nitrate contents (represented by black bars in the heart and grey stripes in liver mitochondria) were plotted on the ordinate axis against arginase activity (Fig. 3). Interestingly, regardless of which tissue it was found, we obtained a steep rise in L-citrulline and nitrate content at low arginase activity followed by a suppression of L-citrulline, nitrite, and nitrate formation with the increase of arginase activity (Fig. 3).

In the literature, there was generally observed that under multiple pathophysiological conditions constitutional NOS activity was suppressed, which was accompanied by elevated ROS production [15-17]. So, it was of interest to find the impact of NG application on ROS production in heart and liver mitochondria and match against arginase activity. For this purpose, we determined the level of hydroperoxide in mitochondria as the most stable of ROS products.

As we observed, under NG treatment there was only a moderate increase in hydroperoxide formation in heart mitochondria, as compared to the steep rise in H_2O_2 production in liver mitochondria (Fig. 4A). Low hydroperoxide formation in heart mitochondria coincided with high mtNOS activity vs. low arginase activity, which was reflected by high L-citrulline and high nitrate content in mitochondria (Figs. 1; 3). However, in liver mitochondria high H_2O_2 production coincided with low L-citrulline and nitrate formation, which indicated low mtNOS activity at much higher arginase activity (Figs. 1; 3). So, an inverse correlation between mtNOS and arginase activity observed in our experiments (Fig. 3) allowed us to build up a dependence of hydroperoxide formation on arginase activity.

As we did above, all arginase activity values were put together and plotted on the abscissa axis; hydroperoxide content (represented by black and gray squares in heart and liver mitochondria respectively) was plotted against arginase activity. In this way, a linear correlation between arginase activity and hydroperoxide production was found over a wide interval of

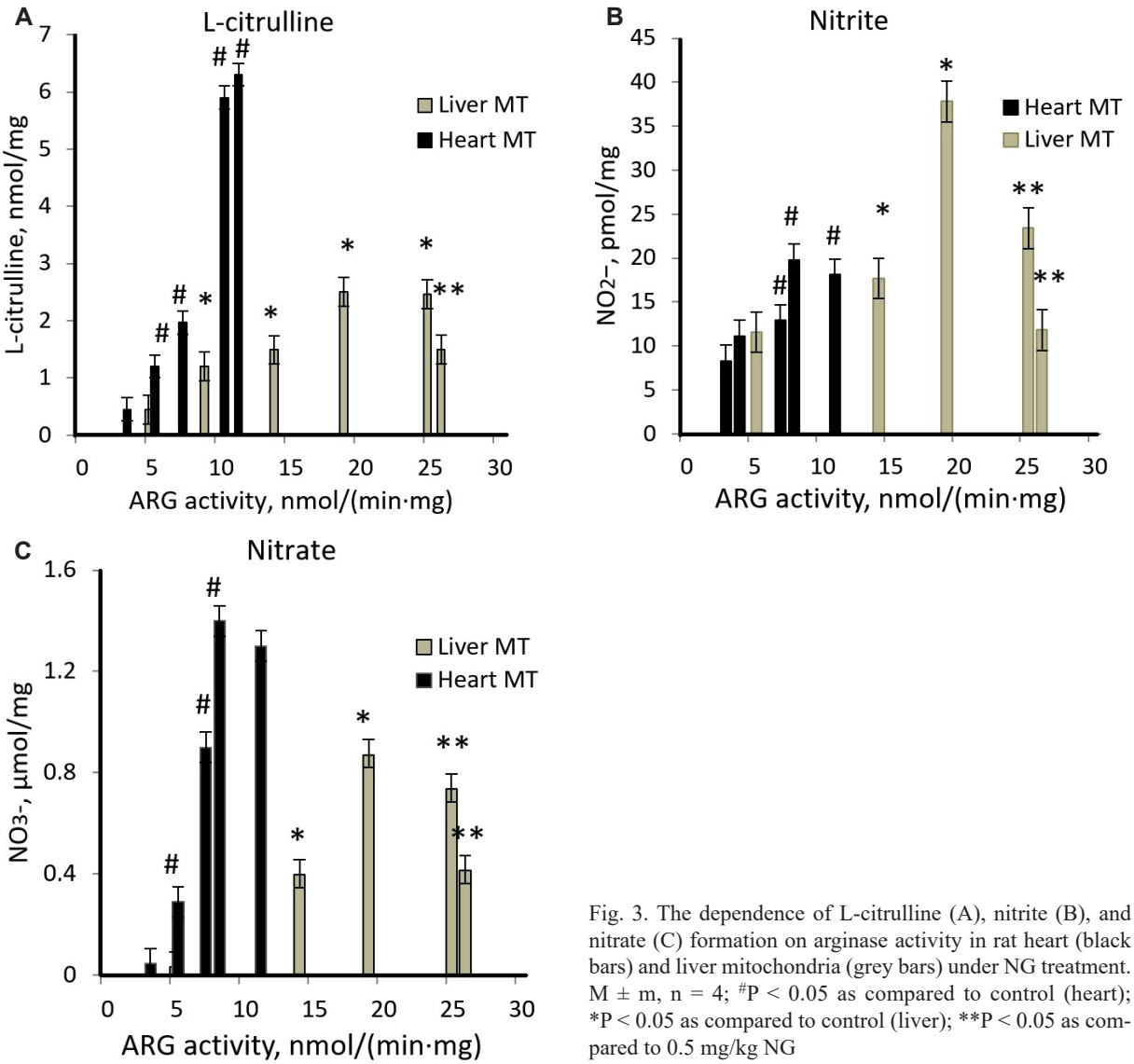

Fig. 3. The dependence of L-citrulline (A), nitrite (B), and nitrate (C) formation on arginase activity in rat heart (black bars) and liver mitochondria (grey bars) under NG treatment. M ± m, n = 4; $^{\#}P < 0.05$ as compared to control (heart); $^{*}P < 0.05$ as compared to control (liver); $^{**}P < 0.05$ as compared to 0.5 mg/kg NG

arginase activities (Fig. 4B). An increase in arginase activity in liver mitochondria resulted in the reduction of mtNOS activity, but in stepwise elevation of ROS production. Thus, the suppression of mtNOS activity caused by arginase activation resulted in a dramatic increase in hydroperoxide formation. Similar effects of ROS overproduction in line with NOS down-regulation were observed by other authors [15, 16]. However, the underlying mechanism of this phenomenon was poorly explained in the literature and still requires much more extensive studies.

DISCUSSION

Based on the analysis of published data, there was often a reciprocal regulation of constitutive NOS and arginase activities under several diseases and pathophysiological conditions including hypertension [15], diabetes [16], and aging [17]. In this work, we have shown that a similar correlation existed between mtNOS and mitochondrial arginase (arginase II), which was observed under NG administration. One plausible explanation of this effect is the limited availability of L-arginine, which is a

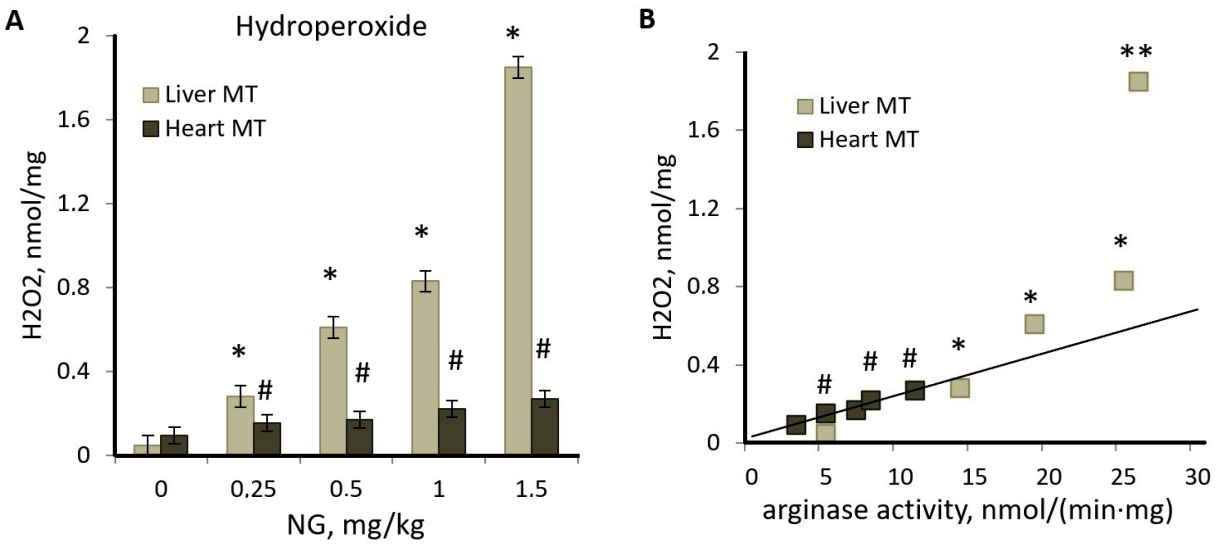

Fig. 4. The dependence of hydroperoxide formation on NG administration in heart and liver mitochondria. A: dose-dependence of H_2O_2 formation on NG application. B: a correlation between arginase activity and hydroperoxide formation. M ± m, n = 4; #P < 0.05 as compared to control (heart); *P < 0.05 as compared to control (liver); **P < 0.05 as compared to 0.5 mg/kg NG

common substrate for both enzymes. Also, as it was shown in the literature, NOS activity could be inhibited by urea, which is an arginase end product [13]. In turn, arginase could be inhibited by NG-hydroxy-L-arginine, which is intermediate product of enzymatic NOS activity [18]. This is the basis for complex reciprocal regulation of NOS and arginase activities involving the regulation of the enzymes by substrate, intermediate, and the end product of enzymatic activity.

In the literature, mtNOS was shown to be activated by mitochondrial Ca^{2+} uptake [23], and earlier we have established a correlation between the stimulation of Ca^{2+} uptake and activation of mtNOS in rat heart and liver mitochondria under the administration of different doses of NG [6]. In this work, the raise in mtNOS activity in both heart and liver mitochondria up to ~0.5-1 mg/kg NG on a whole agreed with the dependence of mtNOS activity on mitochondrial Ca^{2+} uptake [6]. Nevertheless, while similar dose dependence of mtNOS activity on NG was to be expected in both tissues, quite different regulation of mtNOS activity in the heart and liver was found. This motivated us to conduct a comparative study of the regulation of mtNOS and arginase activity, as well as RNS and ROS production, in heart and liver mitochondria.

Based on the experiments, reciprocal regulation of mtNOS and arginase activity in mitochondria was found, and the reciprocal regulation of NO_2^-/NO_3^- content and ROS production, which was dependent on arginase activity (Fig. 3). Interestingly, there was no correlation between mtNOS activity and S-nitrosothiol formation. Possibly, dose-dependent increase in S-nitrosothiols content directly reflected S-nitrosation of mitochondrial proteins under increasing doses of NO delivered from NG [2, 7]. Different of bell-shaped dependence of NO_2^-/NO_3^- formation, a stable increase of S-nitrosothiols content with the increasing dose of NG allow us hypothesize that this process was relatively independent of the mechanisms regulating nitrite and nitrate production under our experimental conditions.

Also, our experiments showed tissue specificity of the interplay between mtNOS and arginase activity as well as RNS and ROS production stimulated by NG in mitochondria. Low arginase activity in heart mitochondria

allowed for the steep rise in mtNOS activity and RNS production in parallel with arginase activation (which indicated the absence of a competition between these enzymes). So, high mtNOS activity agreed with moderate activation of arginase in heart mitochondria, and vice versa, in liver mitochondria mtNOS activity was apparently suppressed by high arginase activity. In line with published data [15-17], suppression of mtNOS activity caused by arginase activation was accompanied by ROS overproduction. As we have shown, there was a correlation between arginase activity and hydroperoxide formation in mitochondria (Fig. 4B).

It is tempting to speculate that in the absence of the competition between the enzymes, low arginase activity in heart mitochondria allowed for much higher mtNOS activation, which in turn could suppress arginase activation. On the contrary, high arginase activity in liver mitochondria led to the suppression of mtNOS activity possibly caused by the competition for physiological substrate, L-arginine, which was accompanied by the elevation of ROS production. It could be hypothesized too that possible depletion of L-arginine due to high arginase activity could result in mtNOS uncoupling, which in turn might contribute to ROS overproduction observed in the experiments.

Observed regularities need more extensive studies for the purpose to find underlying mechanisms of the discussed phenomena. Based on the experiments, we could hypothesize different availability of L-arginine in heart and liver mitochondria under experimental conditions, which resulted in different regulation of mtNOS and arginase activity.

CONCLUSIONS

1. A reciprocal regulation of mtNOS and arginase activities resulted in reciprocal regulation of RNS and ROS production so that high NO_3^- and low H_2O_2 at low arginase activity (heart) *vs.* reduced NO_3^- and elevated H_2O_2 production at high arginase activity (liver) was observed. Thus under NG administration arginase activation resulted in a reciprocal regulation of RNS and ROS production in mitochondria, dependent on the proportion of mtNOS to arginase activity.

2. Suppression of RNS production caused by high arginase activity could result in ROS overproduction. A linear correlation between arginase activity and hydroperoxide formation was found.

3. Observed interplay between mtNOS and arginase activities could represent a cell-specific mechanism of the regulation of RNS and ROS production under physiological and pathophysiological conditions.

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 АКТИВНОСТІ МІТОХОНДРІАЛЬНОЇ NO-СИНТАЗИ ЗА УМОВ ВВЕДЕННЯ НІТРОГЛІЦЕРИНУ В МІТОХОНДРІЯХ СЕРЦЯ І ПЕЧІНКИ ЩУРІВ

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Кардіопротекторний ефект нітрогліцерину реалізується через утворення NO, проте його вплив на метаболізм активних форм азоту (АФА) залишається мало вивченим. Мітохондріальна NO-синтаза (mtNOS) є важливим ендогенним джерелом АФА. Метою нашої роботи було вивчити вплив нітрогліцерину на активність mtNOS і утворення АФА в мітохондріях серця і печінки щурів. У мітохондріях серця активність mtNOS зростала дозозалежно, тоді як в мітохондріях печінки було виявлено лише незначне її підвищення та дзвоноподібну залежність від дози нітрогліцерину. Нітрит- та нітрат-іони, кінцеві продукти перетворення L-аргініну NOS, виявляли також залежність від дози нітрогліцирину. Для пояснення знайдених закономірностей було вивчено вплив останнього на активність аргінази, яка конкурує з NOS за фізіологічний субстрат, L-аргінін. Була встановлена зворотна залежність в регуляції активності обох ензимів. Активність аргінази зростала при введенні нітрогліцерину. Проте в мітохондріях серця спостерігалася висока

активність mtNOS при помірній активації аргінази, тоді як в мітохондріях печінки висока активність аргінази збігалася з пригніченням активності mtNOS за високих доз нітрогліцирину. Низька активність аргінази і висока активність mtNOS у мітохондріях серця корелювали з високим вмістом NO_2^- та NO_3^- і низьким гідропероксиду (H_2O_2), тоді як різка активація аргінази в мітохондріях печінки супроводжувалася зниженням виходу NO_2^-/NO_3^- та одночасним збільшенням генерації H_2O_2. Встановлено лінійну кореляційну залежність між активністю аргінази та продукцією гідропероксиду. Дійшли висновку, що за введення нітрогліцерину активація аргінази призводить до зворотної залежності між утворенням АФА і АФК, зважаючи на співвідношення активностей mtNOS і аргінази. Висока активність аргінази та низька активність mtNOS може призводити до гіперпродукції АФК на тлі пригнічення метаболізму АФА.

Ключові слова: серце; печінка; мітохондрії; mtNOS; аргіназа; активні форми кисню і азоту.

REFERENCES

1. Ignarro LJ, Napoli C, Loscalzo J. Nitric oxide donors and cardiovascular agents modulating the bioactivity of nitric oxide. Circ. Res. 2002;90(1):21-8.
2. Andreadou I, Schulz R, Papapetropoulos R, Turan B, Ytrehus K, Ferdinandy P, Daiber A, Di Lisa F. The role of mitochondrial reactive oxygen species, NO and H_2S in ischemia/reperfusion injury and cardioprotection. J Cell Mol Med. 2020;24:6510-22.
3. Varga ZV, Giricz Z, Liaudet L, et al. Interplay of oxidative, nitrosative/ nitrative stress, inflammation, cell death and autophagy in diabetic cardiomyopathy. Biochim Biophys Acta. 2015;1852:232-42.
4. Radi R, Beckman JC, Bush KM, Freeman BA. Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. Arch Biochem Biophys. 1991;288: 481-7.
5. Reutov VP, Sorokina EG, Ohotin VE, Kositsyn NS. Cyclic transformations of nitric oxide in the organism of mammals. M: Nauka. 1998. [in Russian].
6. Akopova O, Kotsiuruba A, Korkach Y, Kolchinskaya L, Nosar V, Gavenauskas B, Serebrovska Z, Mankovska I, Sagach V. The effect of NO donor on calcium uptake and reactive nitrogen species production in mitochondria. Cell Physiol Biochem. 2016; 39(1):193-204.
7. Bibli S-I, Papapetropoulos A, Iliodromitis EK, et al. Nitroglycerine limits infarct size through S-nitrosation of cyclophilin D: a novel mechanism for an old drug. Cardiovascul Res. 2019;115:625-36.
8. Brookes PS. Mitochondrial nitric oxide synthase. Mitochondrion. 2004;3:187-204.
9. Borutaite V, Brown GC. S-nitrosothiol inhibition of mitochondrial complex I causes a reversible increase in mitochondrial hydrogen peroxide production. Biochim Biophys Acta. 2006;1757:562-6.
10. Ullrich V, Schildknecht S. Sensing hypoxia by mitochondria: a unifying hypothesis involving s-nitrosation. Antioxid Redox Signal. 2014;20:325-38.
11. Nedospasov AA. Competition involving biogenic NO. Biochemistry. 1998;63(7):744-65.
12. Durante W, Johnson FK, Johnson RA. Arginase: a critical regulator of nitric oxide synthesis and vascular function. Clin Exp Pharmacol Physiol. 2007;34(9): 906-11.
13. Wu G, Morris SM. Arginine metabolism: nitric oxide and beyond. Biochem. J. 1998;336:1-17.
14. Elms S, Chen F, Wang Y, Qian J, Askari B, Yu Y, Pandey D, Iddings J, Caldwell RB, Fulton DJ. Insights into the arginine paradox: evidence against the importance of subcellular location of arginase and eNOS. Am J Physiol Heart Circ Physiol. 2013;305(5): H651-H66.
15. Sagach VF, Baziluk OV, Stepanenko LG, Korkach YuP, Kotsuruba AV. ACE inhibitor enalapril action on nitric oxide synthesis, oxidative metabolism, and vascular tone of aging rat. Fiziol Zh. 2007;53(4):15-26. [Ukrainian].
16. Korkach YuP, Kotsuruba AV, Prysiazhna OD, Mogylnytska LD, Sagach VF. NO-dependent mechanisms of ecdysterone protective action on heart and vessels at streptozotocin-induced diabetes mellitus in rats. Fiziol Zh. 2007;53(3):3-8. [Ukrainian].
17. Kotsuruba A, Korkach Yu, Talanov S, Basiluk O, Stepanenko L, Sahach V. Arginase-nitric oxide synthase system changes due to adaptation to swimming in adult and aged rat hearts. Fiziol Zh. 2012;58(1):27-35. [Ukrainian].
18. Buga GM, Singh R, Pervin S, Rogers NE, Schmitz DA, Jenkinson CP, Cederbaum SD, Ignarro LJ. Arginase activity in endothelial cells: inhibition by NG-hydroxy-L-arginine during high-output NO production. Am J Physiol 1996;271:H1988-H98.
19. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrite, nitrate and [15N]nitrate in biological fluids. Anal Biochem 1982;126:131-8.
20. Korkach YuP, Dudchenko NO, Kotsiuruba AV. The role of non-heme iron in the protection effect of ecdysterone on the development of streptozotocin-induced hypoglycemia in rats. Ukr Biochem J. 2008;80(1):46-51.
21. Percival MD, Ouellet M, Campagnolo Ch, Claveau D, Li Ch. Inhibition of cathepsin K by nitric oxide donors: evidence for the formation of mixed disulfides and a sulfenic acid. Biochemistry. 1999;38:13574-83.
22. Huwiler M, Kohler H. Pseudo-catalytic degradation of hydrogen peroxide in the lactoperoxidase/H_2O_2/iodide system. Eur J Biochem. 1984;141:69-74.
23. Dedkova EN, Ji X, Lipsius SL, Blatter LA. Mitochondrial calcium uptake stimulates nitric oxide production in mitochondria of bovine vascular endothelial cells. Am J Physiol. 2004;286:C406-C15.

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