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RHABDOMYOLYSIS ATTENUATES ACTIVITY OF SEMICARBAZIDE SENSITIVE AMINE OXIDASE AS THE MARKER OF NEPHROPATHY IN DIABETIC RATS

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Amine oxidases are involved in the progression of many diseases and their complications, including renal failure, due to the generation of the three toxic metabolites (H_2O_2, aldehydes, and ammonia) in the course of biogenic amines oxidative deamination. The participation of the first two products in kidney pathogenesis was confirmed, whereas the role of ammonia as a potential inducer of the nitrozative stress is not yet understood. The aim of the present study was to test how further intensification of oxidative stress would affect diabetes-mediated metabolic changes. For this purpose, a rat model of glycerol-induced rhabdomyolysis, as a source of powerful oxidative stress due to the release of labile Fe^{3+} from ruptured myocytes, on the background of streptozotocin-induced diabetes was used. The experimental animal groups were as follows: group 1 – 'Control', group 2 – 'Diabetes', group 3 – 'Diabetes + rhabdomyolysis'. A multifold increase in semicarbazide sensitive amine oxidase (SSAO) activity in the kidney and blood, free radicals (FR), MetHb and 3-nitrotyrosine (3-NT) levels in the blood, as well as the emergence of HbNO in plasma and dinitrosyl iron complexes (DNICs) in the liver of animals in group 2 as compared to control were revealed. An additional increase in FR, HbNO levels in the blood, and DNICs in the liver of animals in the diabetes + rhabdomyolysis group as compared to the diabetes group, which correlated with the appearance of a large amount of Fe^{3+} in the blood of group 3 animals, was detected. Unexpectedly, we observed the positive regulatory effects in animals of the diabetes + rhabdomyolysis group, in particular, a decreased SSAO activity in the kidney and 3-NT level in plasma, as well as the normalization of activity of pro- and antioxidant enzymes in the blood and liver compared to animals of diabetes group. These consequences mediated by rhabdomyolysis may be the result of NO exclusion from the circulation due to the excessive formation of NO stable complexes in the blood and liver. The data obtained allow us to consider SSAO activity as a marker of renal failure in diabetes mellitus. In addition, we suggest a significant role of nitrosative stress in the development of pathology, and, therefore, recommend NO-traps in the complex treatment of diabetic complications.

K e y w o r d s: type I diabetes, rhabdomyolysis, renal failure, semicarbazide sensitive amine oxidase, oxidative stress, nitrosative stress.

Elevated levels of amines and polyamines are observed under various pathological conditions that suggests the role of amine oxidases in diseases progression [1]. In particular, semicarbazide sensitive amine oxidase (EC 1.4.3.21; SSAO) is now believed to play a role in renal and vascular diseases, acute and chronic hyperglycemia, and diabetes complications [2] due to the formation of aldehydes and hydrogen peroxide, harmful products of amines oxidative deamination [3, 4] that allows using its level of activity in plasma as clinical marker [2, 5].

In addition to reactive aldehydes and H_2O_2, amine oxidases generate another dangerous product, such as ammonia, which, by our opinion, has not received enough attention. It is important to empha-

size that ammonia is a key component of nitrogen homeostasis, especially in kidney. Renal rate of ammonia synthesis/excretion along with its intrarenal distribution between urine and blood is the main factor in renal acid-base balance regulation. Ammonia is the source of nitrogen for all amino acids. In part, ammonia nitrogen is incorporated into arginine during ornithine cycle in kidney. Arginine synthesis is localized to the proximal convoluted tubule with decreasing activity noted along the proximal straight tubule of kidney [6, 7], then the amino acid released into the renal vein and carried to all tissues and cells [8, 9]. As a consequence, maintenance of plasma arginine levels is a function of its biosynthesis in the kidney. Constant and rather significant quantity of arginine in the body is consumed for the synthesis of nitric oxide (NO) that can be induced in response to various stimuli due to up-regulation of nitric oxide synthases. In addition, arginine also serves as a precursor for the synthesis of polyamines, which in turn, are amine oxidases substrates [10]. NO is a signaling molecule that participates in actually every cellular and organ function in the body. NO, at physiological concentrations, regulates glomerular and medullary hemodynamics, rennin release, tubuloglomerular feedback response, and extracellular fluid volume [11]. Alternatively, NO excessive production can lead to increased formation of peroxynitrite anion, nitration of tyrosine in proteins and production of hydroxyl radicals. Also, it may contribute to pathogenesis of several common renal diseases including diabetic nephropathy [12, 13]. Moreover, at chronic diseases, such as diabetes, stimulated ammoniagenesis is accompanied by kidney hypertrophy that leads to renal injury [14]. Accordingly, elevated levels of ammonia and NO, despite their physiological importance, are toxic and contribute to renal injury and fibrosis. Therefore, increased amount of ammonia, along with other SSAO products, is an important contributor to renal tissue destruction, whereas elevated SSAO activity in kidney can be considered as a marker of pathology.

Earlier, we used animal model of rhabdomyolysis, which is accompanied by powerful oxidative stress due to the release of labile ferric iron (Fe^{3+}) from myoglobin/hemoglobin of ruptured myocytes that led to acute kidney failure. It was demonstrated that all tested amine oxidases, and SSAO to the great extent, play an essential role in the kidney destruction under these conditions [15]. The data formed the basis for the hypothesis on the involvement of the enzyme in the development of other kidney pathologies. STZ-induced diabetes animal model of chronic renal failure also known as diabetic kidney disease (DKD) [16] was the most suitable one for the hypothesis proof. It was important, in our opinion, to further demonstrate the key pathological role of SSAO, as the source of dangerous products, and to found their supposed additive effect on the pathology markers by mechanistic application of two morbid conditions combination.

Thus, we used animal models of chronic kidney disease, namely, diabetes and diabetes complicated with rhabdomyolysis (diabetes + rhabdomyolysis). Taking into account that vascular disorders and liver damage are associated with or may be triggered by kidney abnormalities under diabetes, the level of SSAO activity and markers of nitrosative and oxidative stress in blood, liver and kidney, as targeted tissues, have been estimated.

Materials and Methods

Materials. Streptozotocin, pargiline hydrochloride, methylamine hydrochloride, xanthine oxidase from bovine erythrocytes, were purchased from Sigma ('Sigma', USA), whereas Rat 3-Nitrotyrosine (3-NT) ELISA Kit was from Abbkine, China, (Cat No KTE100307). All other reagents/chemicals were of the highest analytical grade available.

Animals. All manipulations with animals were performed in accordance with European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986) and national requirements for the care and use of laboratory animals. Male Wistar rats weighting approx. 180 g up to 2 months old were maintained on the laboratory pelleted diet and water ad libitum. Vegetable intake as a source of nitrate and nitrite exposure was excluded.

Experimental model. The animal model of a resistant autoimmune Type I diabetes was induced by streptozotocin (STZ), a toxin selective for β cells of the pancreatic islets. Since Wistar rats are not prone to the development of autoimmune diseases, their using allows avoiding irreversible damage of pancreatic islets, as well as toxic effects on regional stem cells and inhibition of regenerative processes in the pancreas, while preserving the possibility of pancreas damage regulation.

The experiment, in which 15 animals were used, included two stages. 5 animals irrespective of treatment were left as control group (intact animals).

Stage 1: Modeling of type 1 diabetes in rats. Type I diabetes in experimental animals was induced by triple intraperitoneale (i.p.) injection of STZ at different doses in 0.5 ml citrate buffer (pH 4.5) at 7 days intervals. For the first and third injections the amount of the drug to be administered was 25 mg/kg animal body weight, while for the second introduction - 20 mg/kg. Each introduction was performed under light anesthesia after 12 h of food deprivation. Incomplete Freud's adjuvant (1 ml) was administered i.p. 1 day prior to the introduction of STZ. A week after the last STZ injection, blood glucose level was determined in blood samples, collected from the tail vein by the tail-tip amputation, using glucometer ('Hlyukofot Plus', Ukraine). This parameter in diabetic rats was (27 ± 8) mmol/l whereas in intact rats it was 6.5 ± 1.7 mmol/l.

Stage 2: Diabetes complicated with rhabdomyolysis. At stage 2, diabetic animals were divided into two groups with about the same blood glucose level (n = 5 per group): diabetic rats ('Diabetes') and diabetic rats with induced rhabdomyolysis ('Diabetes + rhabdomyolysis'). 'Diabetes+rhabdomyolysis' was caused by intramuscular (i.m.) single dose injection of 50% aqueous solution of glycerol (10 ml/kg) into both hind limbs in equal volume 7 days after the last STZ injection. Thus, experimental groups were as follows: group 1 – 'Control', group 2 – 'Diabetes', group 3 – 'Diabetes + rhabdomyolysis'. On the 10^{th} day after glycerol injection animals of all experimental groups were sacrificed.

Sample collection and preparation of rat renal and liver cell fractions. Blood was collected in tubes with and without heparin. Heparinized blood was immediately centrifuged at 1,500 g for 10 min to separate plasma for the further studies. The plasma obtained was stored at -20°C until analysis (up to 24 h). Kidneys and liver were removed on ice. All further steps were performed at 0-4°C. Cell fractions were prepared by differential centrifugation as described earlier [17]. Briefly, organs were homogenized 1:10 (w/v) in 10 mM Tris-HCl buffer, pH 7.2 containing 0.25 M sucrose and protease inhibitors cocktail. The homogenate was filtered through 2 layers of surgical gauze and centrifuged at 700 g for 10 min. The supernatant, centrifuged at 9,000 g for 20 min and at 105,000 g for 1 h, was used for further investigations as cytosolic fraction instantly. The pellet was suspended in the same buffer containing 0.25 M sucrose and centrifuged at 105,000 g for 1 h. The microsomal pellets were resuspended in the same buffer (1 ml per 1 g of wet organ) and used for further investigations immediately.

Determination of 3-nitrotyrosine in plasma proteins. Nitrotyrosine content in plasma proteins was measured by Rat 3-Nitrotyrosine (3-NT) ELISA Kit (Abbkine, China) that is a two-site sandwich one. All procedures were performed according to the manufacturer's protocol. In brief, samples, standards, and blank were applied onto a microplate pre-coated with an antibody specific for 3-NT, and 3-NT present was bound by the immobilized antibody. Afterward, HRP-Conjugate Rat 3-NT detection antibodies were added to the wells. Removal of any unbound sentences or HRP reagent was performed after each incubation step. Following that, chromogen solution was added to the wells, and color developed in proportion to the amount of 3-NT bound in the initial step. The color development was stopped and its intensity was measured colorimetrically.

EPR spectrometry. Liver tissue and blood from each animal were subjected to EPR spectrometry at the temperature of liquid nitrogen using radiospectrometer ('Varian E 109', USA). Instrument settings were as follows: range of the magnetic field - 0–3750 Gs; modulation amplitude of the magnetic field – 8 Gs (2 Gs for free radicals); modulation frequency of the magnetic field – 100 kHz; microwave power 5 mW (0.2 mW for free radicals); microwave frequency (9.6–9.9) GHz.

Enzymatic assays. Assay for SSAO activity. Fluorometric assay designed earlier [15] was used to estimate activity of the amine oxidase in tissue microsomes and blood. In brief, after preincubation of the enzyme preparations with monoamine oxidase inhibitor pargiline (1×10^{-4} M) at room temperature for 20 min, followed by incubation for 30 min at 25°C with substrate (0.1 mM methylamine), the reaction was terminated by addition of 30% $ZnSO_4$. After centrifugation at 12,000 g for 10 min, supernatants were used for detection of H_2O_2 released during enzymatic reaction. One unit of enzyme activity was defined as nmol of product formed per minute per mg protein.

Assay for xanthine oxidase Xanthine oxidase (EC 1.17.3.2; XO) activity was performed according to [18] and expressed as amount of uric acid (UA) produced per min mg protein.

Assay for superoxide dismutase (EC 1.15.1.1; SOD) activity. The superoxide dismutase (SOD) activity was measured in rat erythrocyte hemolysates after chloroform-ethanol treatment [19], kidney and

liver cytosolic fractions using the method of Eriksson and Borg [20] adapted to microtiter plate. The cell contained: sample (~50 µg/ml), sodium bicarbonate buffer (50 mM, pH 10.1), xanthine (2.5 mM), luminol (10 mM). The reaction was initiated with xanthine oxidase (80 µg/ml). The luminol-enhanced chemiluminescence was monitored for 3-5 min at 460 nm λ_{em} with microplate reader FLx800 (Biotek, USA). The amount of protein required to inhibit the rate of superoxide anions induced luminol chemiluminescence reaction by 50%, was defined as 1 unit of enzyme activity.

Assay for catalase (EC 1.11.1.6; CAT) activity. CAT activity was estimated spectrophotometrically following the breakdown of hydrogen peroxide in catalase-peroxide reaction using optical density and molar extinction coefficient at 240 nm as a measure of peroxide concentration. The catalytic process was followed until at least one-half of the substrate was destroyed and activity was expressed as amount of hydrogen peroxide (µmol) broken per min mg protein [21].

Glutathione peroxidase (EC1.11.1.9; GPx) activity. GPx activity was determined with Elman's reagent (DTNB - 5, 5′-dithiobis-2-nitrobenzoic acid) by the amount of reduced glutathione used during the reaction. Optical density was measured at 412 nm using microplate reader µQuant (BioTech, USA). One unit of enzyme activity was defined as µmol of reduced glutathione used per minute per mg protein [22].

Protein concentration. The protein concentration was determined by Bradford method [23] with bovine serum albumin as a standard.

Data analysis. Statistical processing of the results was carried out using Excel 2007 and the STATISTICA 4.5 for Windows software. Shapiro-Wilk test was used for the assessment of normality. Data were analyzed with parametric Student's *t*-test. Results were expressed as mean ± SEM and significance was set at $P < 0.05$.

Results and Discussion

It was important to define the level of SSAO activity in kidney and also in blood and liver as targeted tissues, for renal injury is often accompanied by the development of chronic liver disease and both tissues have an tendency to bleed [24-26].

We observed an assumed increase in values of SSAO activity in kidney and blood (3 fold and 1.4 fold elevation, respectively) and an upward trend in the liver in diabetic rats against the control (Fig. 1). The enzyme activity elevation in plasma (soluble form) is a result of SSAO release from damaged kidney (and liver), and therefore, as was said, can be used as clinical index.

At the same time, myocytes rupture, their content release, and as a consequence powerful oxidative stress induction under diabetes complication with rhabdomyolysis led to unexpected consequences. Specifically, the enzyme activity level was normalized in liver, and significantly decreased in kidney and blood as compared to diabetes and control state, respectively (Fig. 1).

To understand the phenomenon we analyzed, first of all, oxidative stress indexes using EPR analy-

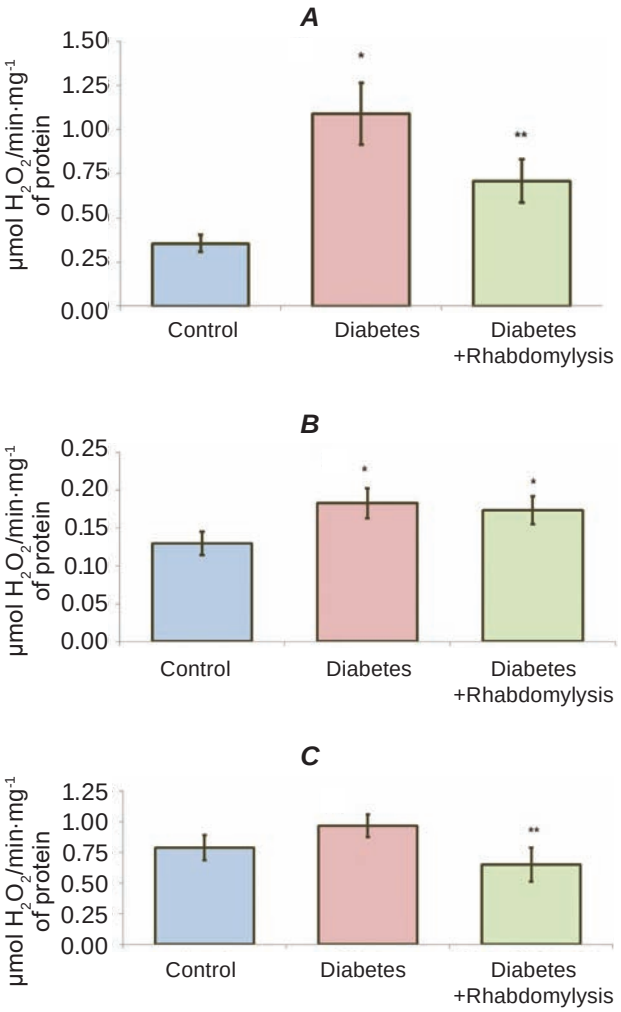

*Fig. 1. SSAO activity in rat kidney (**A**), blood (**B**) and liver (**C**) under streptozotocin-induced diabetes and diabetes+ rhabdomyolysis (Mean ± SD, n = 5 *, to Control, ** to Diabetes, P < 0.05)*

sis as we awaited its leading role in the pathology of diabetes and its complications development. Really, EPR spectra analysis of diabetic rat blood plasma samples revealed oxidative stress evidence, free radicals quantity elevation (1.6 fold) comparing to control (Fig. 2, *A*). Rhabdomyolysis induction in diabetic rats, as expected, additionally stimulated the processes (1.6 fold increase in the number of free radicals compared to 'Diabetes' (Fig.2, *A*)) due to appearance of large quantity of Fe^{3+} that was absolutely absent in intact animals and attended in small amount in diabetic rats (Fig. 2, *B*). The data proved the choice of models and their combination for the experiment was correct but did not confirm their supposed additive effect on the level of SSAO activity as pathology marker.

Antioxidant defenses collectively offset the threat of cell death that could result from the free radical damage, and conversely the redox balance disturbance is a risk factor. Indeed, it was found that pro/antioxidant enzyme activities (XO, SOD and GPx) significantly changed in blood and liver samples of diabetic animals (Fig. 3, *A, B*). The data indicate that additional reason for the stress induction is the elevated superoxide anion generation due to enhancement of XO activity uncompensated with SOD function in liver and, especially, blood (three-fold).

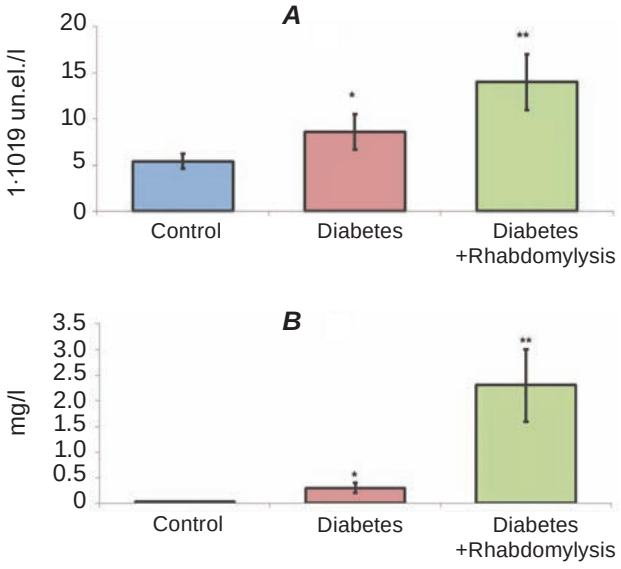

*Fig. 2. EPR investigation of free radicals (**A**) and Fe^{3+} (**B**) levels in rats' plasma samples under streptozotocin-induced diabetes and diabetes + rhabdomyolysis (Mean ± SD, n = 5. *, to Control, ** to Diabetes, P < 0.05)*

At the same time, antioxidant protection in the liver was partially mediated by GPx activity (Fig. 3, *A, B*).

And again, in addition to SSAO activity normalization, following diabetic animals treatment with glycerol we observed decrease of pro- and antioxidant enzymes activities to normal level, except XO activity in kidney (significant 1.7–fold increase) (Fig. 3, *C*). So, we detected pro/antioxidant balance restoring in rats tissues, except for targeted kidney.

Besides, under diabetes+rhabdomyolysis conditions earlier it was found normalization a number of other rat`s oxidative/carbonyl stress parameters as compared to diabetes, such as total aldehydes content and TBA-reactive products in liver, protein CO-groups content in plasma and liver, as well as the degree of skin collagen non-enzymatic glycation which testify to the decrease in accumulation of advanced glycation endproducts (AGEs) on collagen fibers [27].

The question is rising what is the mechanism underlying the normalizing effect of rhabdomyolysis on the biochemical parameters profile under diabetes progression?

As we mentioned above, we presumed the third SSAO end product, ammonium, is potential nitrosative stress inducer due to NO production intensification under the pathology state. But NO, in a free state, has a limited range of action. According to authors [28] 'the half-life of NO in blood ranges from 0.05 to 1.8 ms, whereas lifetimes of ~ 1 μs, 1 ms and 1 s correspond to diffusion distances of ~0.1, 2 and 80 mm, respectively'. However, NO has the ability to form complexes with both heme and nonheme iron and other transition metals, paired thiol groups of proteins, peptides and small molecule compounds such as glutathione and cysteine to form sufficiently stable dinitrosyl complexes with paramagnetic properties. They can be generated via the L-arginine-dependent pathway, catalyzed by the nitric oxide synthases, due to binding of NO as a nitrosyl ligand to a transition metal ion. NO reacts with heme, as well as with iron–sulfur proteins (FeS centers), as well as with proteins containing other transition metals in their active sites, leading to S-, N-, and methemoglobin (MetHb), nitrosyl hemoglobin (HbNO), and dinitrosyl iron complexes (DNICs) formation [29, 30].

Thereby, enhanced NO generation related with SSAO activity elevation under diabetes could lead to formation of the pool of nitrosylated or nitrosated compounds circulating in the body. Really, our data

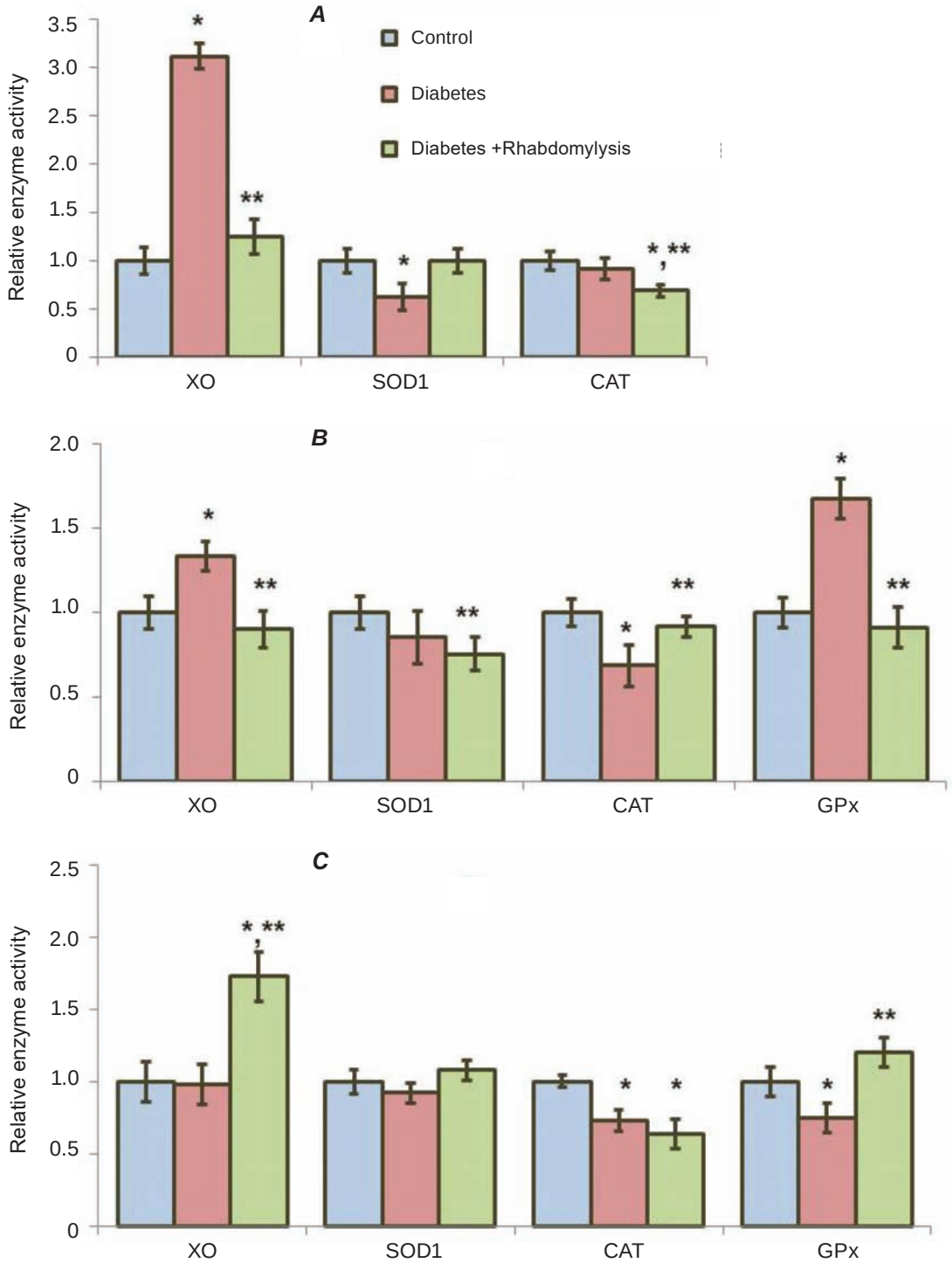

*Fig. 3. Pro- and antioxidative enzymes activities in rat tissues (**A** – blood, **B** – liver, **C** – kidney) under streptozotocin-induced diabetes and diabetes + rhabdomyolysis (Mean ± SD, n = 5, *, to Control, ** to Diabetes, $P < 0.05$)*

revealed under diabetes signs of powerful nitrosative stress, which along with oxidative stress parameters were estimated by EPR spectrometry. Namely, we found almost 2-fold elevation of MetHb, tendency to appearance of HbNO, and 1.8-fold increase in 3-nitrotyrosine level (one of the recognized clinical diabetic marker) in blood samples of diabetic animals compared to control (Fig. 4, *A*, *B*, Fig.5). We have

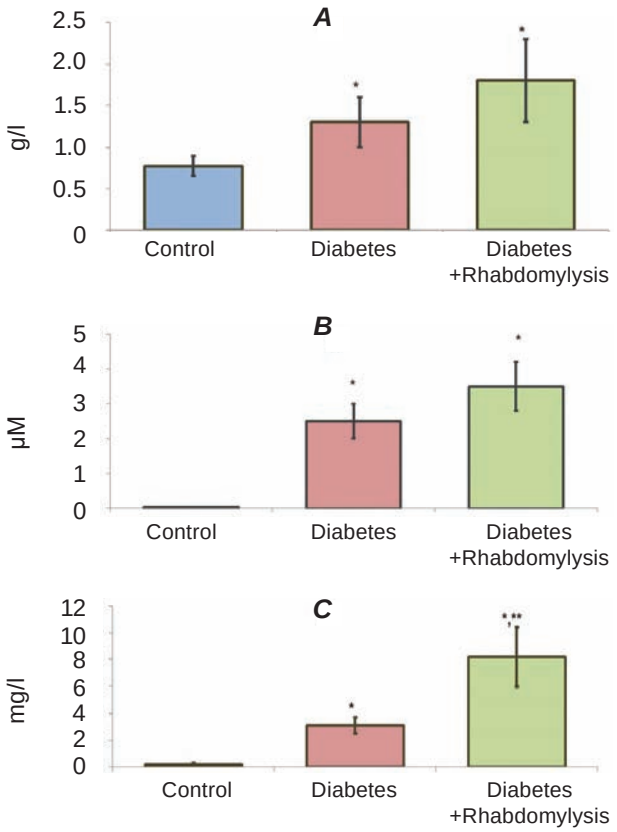

*Fig. 4. EPR investigation of levels of MetHb (**A**) and HbNO (**B**) in blood and DNICs (**C**) in liver samples of experimental rats (Mean ± SD, n = 5, * to Control, P < 0.05*

found also fifteen-fold increase in DNICs (Fig. 4, *C*) and significant drop in the intensity of both mitochondrial signals in liver samples of diabetic animals as compared to control (Table 1). Enzymes that contain iron-sulfur clusters, which are critical for maintaining of viability and functional cell activity, such as guanylyl cyclase, cytochromes P-450 (CYPs-450), enzymes of mitochondrial electron transport chain (ETC), ribonuclease, may be the target of attack by endogenous NO [31]. But we have found other potential NO targets, such as Mn^{2+}, Cu^{2+}- and Mo^{7+}-containing centers, CYPs-450 in the liver remained virtually unchanged (Table).

Usually, the transformation of FeS clusters to DNICs through NO action is highly disruptive that may be a reason for tremendous mitochondrial damage. EPR signals associated with mitochondrial processes correspond to the number of radicals generated in the mitochondria by the ubiquinone and semiquinone sites of the mitochondrial ETC, which are by several orders of magnitude stronger than those associated with oxidative processes [32]. State of the mitochondrial electron transport structures in liver tissue samples can be estimated by the signals of semiquinone free radicals (g = 2.00) and iron-sulfur centers (g = 1.94) that accepted electrons from semiquinones. According to the obtained results (Table), the magnitude of these signals indicates their high inverse correlation with the content of nitrosyl complexes in the blood and liver (r = 0.95-0.98). Thereby, our EPR data show decrease in mitochondrial quantity of free radicals (1.33-fold) and iron-sulfur centers (1.21-fold) (Table) that are the source of DNICs in liver of diabetic rats compared to control and reflect the significant decrease in the rate of electron transfer in the mitochondrial ETC under the pathology.

Such transformations have been detected in many different iron-sulfur proteins and usually inhibit the enzymatic activity of the affected protein. But the process is reversible because complex cellular systems have evolved to compensate the danger of NO damage [33]. So, MetHb reduction systems, predominantly the NADH-cytochrome b5 reductase 3 (NADH-methemoglobin reductase) pathway,

Table. EPR parameters of rats' liver samples under streptozotocin-induced diabetes and diabetes+ rhabdomyolysis (Mean ± SD, n = 5 to Control, P < 0.05)*

EPR parameters, rel. un.	Group		
	Control	Diabetes	Diabetes + rhabdomyolysis
CYPs-450	1.0 ± 0.2	1.2 ± 0.3	0.97 ± 0.30
Mn^{2+}-containing centers	1.0 ± 0.1	1.0 ± 0.2	0.97 ± 0.21
Cu^{2+}-containing centers	1.0 ± 0.2	0.89 ± 0.23	0.87 ± 0.22
Mo^{7+}-containing centers	1.0 ± 0.1	1.2 ± 0.3	0.95 ± 0.21
Mitochondrial free radicals	1.0 ± 0.1	0.75 ± 0.16*	0.67 ± 0.12*
Mitochondrial iron-sulfur centers	1.0 ± 0.1	0.82 ± 0.18*	0.76 ± 0.13*

normally limit its accumulation. Protein FeS centers also are repaired or replaced [30]. The active repair of DNICs appears to be a general process that helps counteract the toxicity of NO exposure. Besides, reversible formation of sufficiently stable HbNO and DNICs ensure storage and transfer of NO molecules in cells and tissues and under diabetes toxic action of NO should be extended by NO radical. This balance depends on oxygen status, and MetHb level because if the MetHb level increases to more than 30% of total hemoglobin, symptoms of hypoxia occur [30, 34, 35].

Renal dysfunction in the case of additional rhabdomyolysis application may be contributing factor to methemoglobinemia (increase in MetHb) that results in decrease of oxygen delivery to the tissues and strong hypoxia development. Really, we have found under this combined pathological state tendency to elevation of MetHb quantity and HbNO level in blood, and sufficient DNICs increase in the liver samples (2.6-fold) compared to diabetes. But enhanced DNICs and HbNO are stable under hypoxia thereby these complexes cannot release NO in a free state that explains the decrease of tyrosine nitration of proteins to normal level under rhabdomyolysis application (Fig. 5). It may be the main reason of the normalizing action of rhabdomyolysis conditions on some biochemical parameters, including SSAO activity, under diabetes progression. Under this combined pathological state further drop in the intensity of both mitochondrial signals (mithochondrial free radicals and iron-sulfur centers quantity) was observed, whereas other potential NO targets were still unchanged (Table). It is the evidence of degradation of mitochondrial ETC iron-sulfur proteins by nitric oxide, which is the main target under diabetes + rhabdomyolysis state.

Taken together, the data obtained allow considering the SSAO activity level as a marker of

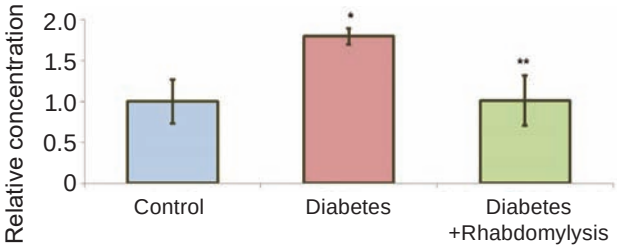

*Fig. 5. 3-Nitrotyrosine level in plasma of experimental animals (Mean ± SD, n = 5, *to Control, ** to Diabetes, P < 0.05)*

renal failure under system diseases such as diabetes and its complications.

We also supposed substantial role of nitrosative stress in the pathology development, and NO-trapping agents (ferric iron in the case) as potential therapeutics for diabetic complications care.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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РАБДОМІОЛІЗ ПОСЛАБЛЮЄ АКТИВНІСТЬ СЕМІКАРБАЗИД-ЧУТЛИВОЇ АМІНОКСИДАЗИ ЯК МАРКЕРА НЕФРОПАТІЇ У ДІАБЕТИЧНИХ ЩУРІВ

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Амінооксидази беруть участь у розвитку багатьох захворювань та їх ускладнень, зокрема ниркової недостатності, через утворення трьох токсичних метаболітів (H_2O_2, альдегідів та амонію) в реакціях окисного дезамінування біогенних амінів. Участь перших двох продуктів у патогенезі нирки наразі переконливо доведено, тоді як роль амонію, як потенційного індуктора нітрозативного стресу, залишається нез'ясованою. Метою цього дослідження було перевірити, як подальше посилення окисида-

тивного стресу вплине на метаболічні зміни, зумовлені цукровим діабетом. Для цього було використано модель гліцерол-стимульованого рабдоміолізу у щурів, як джерела потужного оксидативного стресу внаслідок вивільнення лабільного Fe^{3+} із зруйнованих міоцитів, на тлі стрептозотоцин-індукованого діабету. Було сформовано наступні експериментальні групи: 1 група – «Контрольна», 2 група – «Діабет», 3 група – «Діабет + рабдоміоліз». У тварин 2-ї групи порівняно з контролем спостерігалось багаторазове підвищення активності семікарбазидчутливої аміноксидази (SSAO) в нирці і крові, рівня вільних радикалів (ВР), MetHb і 3-нітротирозину (3-NT) в крові, а також поява HbNO в плазмі та динітрозильних комплексів заліза (ДНКЗ) в печінці. У тварин групи «Діабет+рабдоміоліз» порівняно з групою «Діабет» продемонстровано додаткове підвищення рівня ВР, HbNO в крові та ДНКЗ в печінці, що корелювало з появою в крові тварин 3-ї групи великої кількості $Fe^{3+,}$ яке було повністю відсутнє у інтактних тварин. Несподівано, у тварин групи «Діабет+рабдоміоліз» ми виявили позитивні регуляторні ефекти порівняно з групою «Діабет», зокрема, зниження активності SSAO в нирці та рівня 3-NT у плазмі, а також нормалізацію активностей про- та антиоксидантних ензимів в крові та печінці. Такі наслідки, опосередковані рабдоміолізом, можуть бути результатом виключення NO з кровообігу тварин через надмірне утворення його стабільних комплексів в крові та печінці. Отримані дані дозволяють розглядати активність SSAO як маркер ниркової недостатності за цукрового діабету. Крім того, ми припускаємо значну роль нітрозативного стресу у розвитку даної патології, на основі чого можемо рекомендувати пастки NO в комплексному лікуванні ускладнень діабету.

Ключові слова: цукровий діабет I типу, рабдоміоліз, ниркова недостатність, чутлива до семікарбазиду аміноксидаза, окислювальний стрес, нітрозативний стрес.

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