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Blockade of L-type calcium channels alters hepatic mitochondrial function in insulin-resistant rats

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Mitochondria are central organelles in maintaining energy and oxidative homeostasis. Despite intensive research, the function of mitochondria and the mechanisms of its regulation under physiological conditions and in insulin resistance require detailed investigation. The aim of this study was to investigate the effect of blockade L-type calcium channels in insulin-resistant rats on hepatic mitochondrial oxidative function and changes in its oxidative status. Insulin resistance was modeled in 6-month-old male Wistar rats by 14 days of high-fat feeding. Standard fed animals served as controls. Verapamil was administered intraperitoneally at a dose of 1 mg/kg to block L-type calcium channels. Indicators of pro- and antioxidant systems (active products of tiobarbituric acid, reduced glutathione, catalase, Cu,Zn-superoxide dismutase) were assayed in the liver homogenate extracted from anesthetized animals after 3 h. Mitochondrial function was studied by the Chance polarographic method using different metabolic substrates. It was shown that in intact animals blockade of L-type calcium channels reduced the efficiency of mitochondrial respiration (V3/V4) in liver mitochondria during oxidation of all substrates through an inhibitory effect on the phosphorylation respiration (V3) and a stimulatory effect on the controlled respiration (V4), without affecting the oxidative status of the liver. In rats with insulin resistance the rate of V3 during oxidation of both NAD- and FAD-dependent substrates was decreased, violations of oxidative status and increased antioxidant protection were detected. However, in insulin-resistant rats blockade of L-type calcium channels significantly enhanced basic respiration (V2) during NAD-dependent substrate oxidation, V3 and V4 during palmitoyl lipid substrate oxidation, reduced the V3/V4 ratio compared to control, and partially or fully restored the violation of the oxidative status. This may indicate the involvement of calcium mechanisms in the disturbance of the oxidative status of the liver and the regulation of energy metabolism in mitochondria during insulin resistance.
Key words: calcium channels blockade; insulin resistance; liver; mitochondria; energy metabolism.

INTRODUCTION

The liver plays a central role in the regulation of carbohydrate and lipid metabolism and is one of the organs that directly exerts an effector and regulatory response in conditions of insulin resistance [1]. Insulin has both a direct effect on the liver through the regulation of glycogenolysis and gluconeogenesis, and an indirect effect through the regulation of lipogenesis in white adipose tissue. Insulin resistance as a result of obesity or lipid overload leads to liver damage by both mechanisms [1, 2]. An indirect mechanism is endoplasmic reticulum damage caused by excessive free fatty acid delivery to hepatocytes. In addition, insulin resistance causes inflammation through activation of Kupffer cells leading to further liver damage and may be a base for the development of metabolic syndrome, non-alcoholic fatty liver disease, or type II diabetes [2, 3].

Reactive oxygen species (ROS) which production in mitochondria increases with hyperglycemia, high levels of free fatty acids, an imbalance between pro- and antioxidants etc. [4], also have a damaging effect on liver cells in the metabolic syndrome. The antioxidant

enzyme system (AES), which is represented in the body by several levels of protection and includes enzymatic and non-enzymatic chains, counteracts the negative effects of active oxygen metabolites. AES is able to reduce the intensity of free radical processes by neutralizing free radicals (ROS). The key enzymes of ROS catabolism are superoxide dismutase, catalase and tripeptide glutathione [5].

In addition to the endoplasmic reticulum, mitochondria are also affected under conditions of insulin resistance, at least in skeletal muscle and white adipose tissue, according to Sangwung, et al. [6]. However, the question of the effect on liver mitochondria remains poorly understood. Mitochondria are functionally linked to the endoplasmic reticulum. Therefore, under conditions of damage to the endoplasmic reticulum, changes in mitochondrial function would be expected, including those related to the regulation of intracellular calcium concentration [7]. However, changes in liver mitochondrial function in insulin resistance and their relationship to changes in calcium signaling have not been adequately investigated.

Despite the widespread use of L-type Ca^{2+} channel blockers in clinical practice, there are conflicting data regarding their effect on carbohydrate and lipid metabolism, insulin resistance, and liver function in healthy subjects and in the conditions of metabolic syndrome and type I and type II diabetes development [8, 9]. According to some authors, insulin resistance increases when calcium channels are blocked in patients with type II diabetes, but not in healthy subjects [9]. Other data show that calcium channel blockade may even lead to insulin resistance [8]. However, there is no doubt that alterations in intracellular calcium homeostasis are involved in the regulation of insulin synthesis and intracellular signaling, as well as in the myogenic and neuronal regulation of portal blood flow, thus affecting hepatocellular and mitochondrial functional status [9].

It is known that L-type Ca^{2+} channels are present in myocardium [7], vascular smooth muscle cells [8], and the autonomic nervous system, where is inhibitory effect by L-type Ca^{2+} channels blockers [10]. Blockers of L-type Ca^{2+} channels regulates the activity of the adrenal medulla [11] and pancreatic β-cells [12], which express these channels. Such channels have not yet been found in hepatocytes. Calcium channels blockade (CCB) may indirectly affect metabolic processes in the liver by inhibiting the work of the autonomic nervous system and the cardiovascular system, as well as by altering humoral regulation through a decrease of releasing insulin and products of the enterochromaffin cells. However, there is no doubt that alterations in intracellular calcium homeostasis are involved in the regulation of insulin synthesis and intracellular signaling, as well as in the myogenic and neuronal regulation of portal blood flow, thus affecting hepatocellular and mitochondrial functional status [1, 8, 12].

Consequently, the aim of our study was to investigate the effect of L-type calcium channel blockade on hepatic mitochondrial oxidative function and pro/antioxidant status in insulin-resistant rats.

METHODS

Animal experiments were conducted according to the requirements of the European Convention for the Protection of Vertebrate Animals and the Law of Ukraine No. 3447-IV "On Protection of Animals from Cruelty".

Male Wistar rats aged 6 months (300-350 g) were used in the study. The animals were divided into 4 groups (n = 5): 1) control – intact animals, 2) CCB – rats with injection of L-type calcium channel blocker, 3) HFD – rats with insulin resistance modeling by consumption of a high-fat diet (HFD), 4) HFD+CCB – animals with HFD and injection of L-type calcium channel blocker. Insulin resistance was modeled by feeding animals a high-fat diet containing 58% animal fat of total calories [13, 14] for 14 days of the experiment. To block L-type calcium channels, verapamil was administered intraperitoneally at

1 mg/kg bw 3 h before sampling [15].

Liver fragments removed from anesthetized rats with urethane (1.5 g/kg) were immediately placed on ice and homogenized with the addition of 0.9% cold KCl solution (4°C). We determined the content of secondary products of lipid peroxidation reacting with 2-thiobarbituric acid (TBA-active products), the content of reduced glutathione, the activity of catalase and Cu,Zn-superoxide dismutase (SOD) in the liver homogenates according to generally accepted methods [16].

The mitochondrion isolation buffer (mmol/l): KCl - 120, HEPES - 10, K_2CO_3 - 2, EGTA-1 (pH 7.2), and the incubation buffer – KCl - 120, HEPES - 10, K_2CO_3 - 2, KH_2PO_4 - 2 (pH 7.2). The Chance polarographic method [17, 18] was used to study the processes of respiration and oxidative phosphorylation in mitochondria. The following substrates of oxidation were used (mmol/l): 1) succinate (Sc) - 3.5; 2) glutamate - 3 and malate - 2.5 (G+M); 3) pyruvate - 3 and malate - 2.5; 4) palmitoyl-DL-carnitine - 2.5 and malate - 2.5 (Pm+M). Oxidative phosphorylation was stimulated by the introduction of 200 μmol/l ADP. The following mitochondrial oxygen consumption parameters were recorded: state 2 or V2 (oxygen consumption before the addition of ADP), state 3 or V3 (oxygen consumption stimulated by ADP), and state 4 or V4 (oxygen consumption after the cessation of ADP phosphorylation). The respiratory control ratio (RCR) or V3/V4 described by Chance and Williams was measured [17, 18].

Results are expressed as Mean ± SD. All variables were tested for normal distribution using the Kolmogorov-Smirnov and Lilliefors tests ($P > 0.05$). Homogeneity of variance was checked using the two-way ANOVA test and post-hoc Tukey's test. Differences were considered significant at $P < 0.05$. All statistical calculations were performed on GraphPad Prism 8 software.

RESULTS

The results of this study are indicative of a violation of the redox balance in the liver of the experimental animals. Maintenance of animals on a high-fat diet for 2 weeks resulted in a significant 14% increase in the content of TBA-AP in the liver of rats compared to the control, indicating the intensification of ROS generation processes. In addition, a 28% decrease in the content of reduced glutathione, a 64% increase in the activity of the anti-peroxide enzyme catalase, and a trend toward an increase in the activity of SOD were observed in these group.

Blockade of L-type Ca^{2+}-channels did not affect the redox balance in intact rats. However, significant changes in liver pro- and antioxidant homeostasis were observed in animals treated with HFD. In particular, probably due to the effect of the blockade, the level of TBK-AP decreased, practically normalizing to the level of the control group, and the levels of reduced glutathione and catalase were partially restored. Catalase activity probably stayed higher than in intact animals (Table 1).

The effect of high-fat diet on ADP-stimulated respiration led to a significant decrease in the oxidative function of mitochondria: the level of V3 decreased during oxidation of Sc (43.12%), G+M (42.62%) and P+M (38.02%). V4 ratio had no significant changes in mitochondrial energization during oxidation Sc and G+M. But there was a significant decrease in mitochondrial functioning with P+M (31.68%) and a significant increase with Pm+M (30.45%). Changes of RCR (V3/V4 ratio) indicated a significant decreasing of the mitochondrial respiratory function compared to the control: Sc (51.47%), G+M (54.65%), P+M (34.3%) and Pm+M (40.11%). This is due to the decreasing of V3 during oxidation of Sc, G+M and P+M, and the increasing of V4 during the oxidation of Pm+M. It is interesting that V2 tended to increase during oxidation of Sc (20.5%), G+M (28.5%) and P+M (28.9%) and significantly increased during oxidation of Pm+M (32.2%) (Table 2; 3).

Under L-type calcium channels blockade ADP-stimulated oxidation (V3 ratio) didn`t changed with all oxidative substrates. At the same time the V4 ratio increased by during oxi-

Table 1. Indicators of pro-/antioxidant system in rat liver with insulin resistance under the conditions of CCB (M ± SD)

Indicator	Control	HFD	CCB	CCB+HFD	HFD effect	CCB effect	Interaction
TBA-AP, μmol/ml	24.04 ±0.76	27.53±2.09*	23.76±1.06	24.68±0.89**	F = 11.34 P = 0.0056	F = 5.744 P = 0.0337	F = 3.875 P = 0.0725
GSH, μmol/mg	4.85±0.49	3.47±0.63*	5.11±0.61	4.01±0.41	F = 21.05 P = 0.0006	F = 2.182 P = 0.1654	F = 0.2673 P = 0.6146
CAT, μmol/mg	3.10±0.54	5.06±0.74*	2.98±0.12	4.85±0.27*,**	F = 64.34 P < 0.0001	F = 0.4793 P = 0.5019	F = 0.03319 P = 0.8585
SOD, μmol/mg	13.68±0.69	14.94±0.76	13.99±0.69	13.61±1.22	F = 1.039 P = 0.3282	F = 1.406 P = 0.2587	F = 3.601 P = 0.0821

*P < 0.05 compared to control; **P < 0.05 between CCB and HFD+CCB.

dation of FAD-generated substrate Sc (31.8%), and there was a tendency to increase NAD-generated G+M (28.1%) and Pm+M (27.2%). RCR significantly decreased during oxidation all substrates with the due to the V4 increasing. The V2 ratio tended to increase during oxidation of all substrates except Pm+M.

In contrast to control rats, blockade of calcium channels in insulin-resistant rats caused a V3 ratio decreasing during the oxidation of Sc (57.7%), G+M (44.6%) and Pm+M (48.32%), and a tendency to decrease V3 ratio during P+M oxidation (26.63%).

V4 ratio changed in the opposite way to the control group: it decreased significantly during the oxidation of P+M (38.4%) and had a tendency to decrease during the oxidation of Sk (15.85%) and Pm+M (26.98%). The V3/V4

Table 2. Oxidative function of rat liver mitochondria using the metabolic substrates glutamate and malate, succinate (M ± SD)

Oxidative substrate	Control	HFD	CCB	HFD+CCB	HFD effect	CCB effect	Interaction
Glutamate and malate							
V2, nmol/ $mg^{-1}\times min^{-1}$	6.52±1.61	8.38±2.40	8.70±2.08	4.75±0.85**,***	F=1.400 P=0.2564	F=0.6740 P=0.4254	F=10.82 P=0.0054
V3, nmol/ $mg^{-1}\times min^{-1}$	28.44±5.38	16.32±4.44*	24.13±9.68	15.75±5.32*	F=10.63 P=0.0062	F=0.6054 P=0.4505	F=0.3541 P=0.5620
V4, nmol/ $mg^{-1\times}min^{-1}$	5.68±1.31	6.85±0.58	7.90±2.17	4.78±1.05**	F=2.105 P=0.1689	F=0.01102 P=0.9179	F=10.11 P=0.0067
RCR V3/V4	5.05±0.68	2.29±0.68*	3.04±0.76*	3.24±0.44*	F=18.34 P=0.0005	F=3.162 P=0.0933	F=24.29 P=0.0001
Succinate							
V2, nmol/ $mg^{-1}\times min^{-1}$	7.55±1.60	9.10±1.43	10.2±2.32	6.30±0.73**	F=2.248 P=0.1576	F=0.009161 P=0.9252	F=12.09 P=0.0041
V3, nmol/ $mg^{-1}\times min^{-1}$	36.50±8.85	20.76±5.95*	29.78±2.88	15.25±5.12*,***	F=26.10 P=0.0002	F=4.269 P=0.050	F=0.04207 P=0.8407
V4, nmol/ $mg^{-1}\times min^{-1}$	6.16±0.62	7.90±1.52	9.03±1.26*	6.50±1.36**	F=0.4500 P=0.5141	F=1.567 P=0.2327	F=13.28 P=0.0030
RCR V3/V4	5.09±0.53	2.47±0.61*	3.33±0.42*	2.40±0.89*	F=35.24 P<0.0001	F=9.342 P=0.0085	F=8.006 P=0.0134

Note: here and in Table 3. *P < 0.05 compared to control; **P < 0.05 between CCB and HFD+CCB; ***P < 0.05 between HFD and HFD+CCB.

ratio significantly decreased during oxidation of NAD-generated substrates compared to the control: G+M (35.8%), Pm+M (38.43%) and during oxidation of FAD-generated Sc (52.84%). There were no significant changes of V3/V4 ratio during oxidation of P+M (Table 2; 3).

There was a difference in the effect of CCB with high-fat diet and without high-fat diet. According to the ANOVA test, the V4 ratio was increased in the high-fat diet group. Therefore, a decrease of V3/V4 ratio was observed. The CCB effect on V4 and V3/V4 ratio was similar to the effect of HFD. However, V4 ratio decreased compared to the control during the interaction of HFD and CCB. by interaction of HFD and CCB V2 ratio was decreased while under the action of CCB without HFD there are tendencies to increasing the mitochondrial oxidative function with all substrates, except Pm+M. V3 ratio was independent of HFD only during oxidation of Pm+M and it depends of HFD during oxidation of Sc, G+M and P+M. Consequently, changes in RCR are consistent with changes in V3 and V4 ratio (Table 2; 3).

Thus, there is an inhibition of oxidative phosphorylation with the interaction of HFD and CCB, which is appeared during the oxidation of succinate (II complex of the electron transport chain) and palmitoyl (I complex of the electron transport chain). At the same time, the use of CCB without HFD in rats does not lead to significant changes in the oxidative function, while the use of HFD suppresses the level of mitochondrial energization on all respiratory substrates, except for palmitoyl. In the latter case, an activation of the oxidative function of the mitochondria is observed.

The level of TBA-AP is indirectly dependent on CCB and does not change under the conditions of CCB on the background of HFD. In contrast, the activity of antioxidant system is independent of CCB and is activated by HFD.

DISCUSSION

The data obtained indicate changes in liver oxidative phosphorylation during insulin resistance and differences in the regulatory

Table 3. Oxidative function of rat liver mitochondria using the metabolic substrates pyruvate and malate, palmitoyl and malate (M ± SD)

Oxidative substrate	Control	HFD	CCB	HFD+CCB	HFD effect	CCB effect	Interaction
Piruvate and malate							
V2, nmol/ $mg^{-1}*min^{-1}$	6.23±1.21	7.98±2.28	8.50±3.02	3.98±0.61*,**,***	F=2.519 P=0.1320	F=0.9841 P=0.3360	F=12.90 P=0.0024
V3, nmol/ $mg^{-1}\times min^{-1}$	22.83±1.94	14.15±2.30*	18.75±3.48	16.75±5.74	F=4.019 P=0.0647	F=0.1980 P=0.6632	F=4.019 P=0.0647
V4, nmol/ $mg^{-1}\times min^{-1}$	8.05±1.38	5.50±1.16*	7.38±1.89	5.33±0.82*	F=12.29 P=0.0035	F=0.4197 P=0.5276	F=0.1452 P=0.7089
RCR V3/V4	3.79±0.29	2.49±0.58*	2.59±0.37*	3.12±0.78	F=2.070 P=0.1738	F=1.178 P=0.2975	F=12.04 P=0.0041
Palmitoyl and malate							
V2, nmol/ $mg^{-1}\times min^{-1}$	7.08±1.07	9.36±1.16*	6.20±1.85	4.43±0.80*,***	F=0.1796 P=0.6786	F=6.553 P=0.0237	F=11.58 P=0.0047
V3, nmol/ $mg^{-1}\times min^{-1}$	22.56±2.20	27.12±6.14	20.75±6.15	11.66±2.01*,***	F=0.9707 P=0.3440	F=14.15 P=0.0027	F=8.800 P=0.0118
V4, nmol/ $mg^{-1}\times min^{-1}$	5.30±1.26	7.62±1.26*	7.28±1.19	3.87±0.68**, ***	F=0.8585 P=0.3724	F=2.292 P=0.1559	F=23.78 P=0.0004
RCR V3/V4	5.36±0.11	3.21±0.63*	2.86±0.79*	3.30±1.13*	F=5.325 P=0.0381	F=10.45 P=0.0065	F=12.04 P=0.0041

effect of L-type calcium channel blockade on mitochondrial functions and maintenance of pro- and antioxidant homeostasis under conditions of lipid overload (HFD) and during normal diet. Measurements of hepatic mitochondrial function during the oxidation of various metabolic substrates indicate that Ca^{2+}-dependent mechanisms are involved in regulating mitochondrial oxidative function through both the I and II complexes of the electron transport chain.

Changes in hepatic mitochondrial function and redox status in insulin resistance. The development of insulin resistance induced by a high-fat diet, as shown by us in this and previous studies [13, 14], was accompanied by an intensification of lipid peroxidation (LPO) processes, stressing the antioxidant defense processes (catalase and SOD activity) and reducing their reserves (reduced glutathione). The obtained results indicate a violation of the redox balance in the liver of experimental animals.

These results are consistent with literature data on the activation of antioxidant systems under conditions of insulin resistance. It has been shown [4] that such oxidative stress is associated with increased free fatty acids and hyperglycemia, which determine the cytotoxic effect in type II diabetes and the preceding insulin resistance.

Increased ROS production by mitochondria may be a consequence of their dysfunction. The results we obtained indicate that in rats consuming HFD, the oxidative function of mitochondria is inhibited due to decreasing in the rate of active respiration (V3), which in turn is associated with inhibiting the work of complexes I and II of the respiratory chain. In insulin-resistant rats inhibition of mitochondrial energetics was observed during oxidation of NAD-dependent (H+M and P+M) and FAD-dependent (Sc) substrates, whereas oxidation of NAD-dependent lipid substrate (Pm+M) increased.

It is well known [19] that the respiratory function and the synthesis of ATP in mitochondria are inhibited in type II diabetes mellitus (DM). Experimental and clinical studies have shown that the rate of ADP-stimulated respiration of isolated mitochondria is significantly decreased in diabetes [20], which is accompanied by an increase in the $NADH/NAD^+$ ratio due to a pronounced dysfunction of complex I of the respiratory chain, as well as a decrease in the activity of complexes II, IV, V (ATP synthase) [6]. According to Oliveira et al [21], the level of controlled respiration decreased during oxidation of the FAD-dependent substrate succinate in liver mitochondria of rats with experimental nonalcoholic fatty liver disease (NAFLD). It was demonstrated [4] that fatty acid β-oxidation was suppressed in the liver of rat with NAFLD in the presence of increased NADH/NAD. It is worth noting that NAFLD occurs as a result of the development of insulin resistance, which is accompanied by early degenerative changes in liver metabolism [22]. Summary, insulin resistance is an important contributor to impaired energy metabolism in metabolic syndrome, type II diabetes and obesity.

The role of L-type calcium channels in the regulation of mitochondrial function and redox status in rat liver. In our study we showed that blocking L-type calcium channels did not have a significant impact on oxygen consumption and oxidative homeostasis in liver mitochondria. In particular, no significant changes in mitochondrial energization were observed for all respiratory substrates during active and controlled breathing, although the V3/V4 ratio (respiratory efficiency) decreased with the use of an L-type calcium channel blocker.

It is known that CCB can have an indirect effect on the liver via the myocardium and vascular cells [8, 9], by inhibiting the entry of Ca^{2+} into cardiomyocytes and arterial smooth muscle cells. So, this has an effect on the pumping function of the heart and on the tone of the blood vessels [9]. CCB also affects the tone of the autonomic nervous system, due to the sympathoinhibitory effect [10]. L-type

calcium channels blockade also has impact on secretory processes involved in the regulation of liver function: it suppresses adrenaline secretion by enterochromaffin cells [11] and insulin secretion by beta cells [12]. Besides indirect effects, direct effects of CCB on the liver cannot be excluded. Although hepatocytes do not have potential-gated calcium channels on their membrane and therefore a direct influence of CCB on its functional state is impossible, there are three types of liver cells that participate in the regulation of intrahepatic blood flow and contain slow L-type calcium channels on their membrane: smooth muscle cells (portal vein, central and hepatic venules and hepatic arterioles), Kupffer cells and stellate cells [23, 24]. The latter are considered as the main regulator of sinusoidal blood flow and exchange processes between the extracellular environment and the space of Disse. In addition, they are probably involved in the transduction of nerve signals to liver parenchymal cells [23]. Therefore, the effect of CCB on hepatocyte mitochondria may be mediated by its action on one of these cell types, most likely hepatic stellate cells.

From the above, it can be concluded that CCB affects the energy metabolism of the liver not only systemically, but also locally through the cellular elements of the liver. This is confirmed by the suppression of mitochondrial oxidative function on I and II complexes of the respiratory chain when L-type calcium channels blockade are involved. Decreased mitochondrial respiratory efficiency in the presence of CCB may be associated with uncoupling oxidative phosphorylation. As a result, mitochondria produce less ATP and the energy metabolism of the cell decreases with the same amount of delivered oxygen.

The role of L-type calcium channels in the regulation of rat liver mitochondrial function and redox status in insulin resistance. Insulin resistance induced by a high-fat diet not only significantly affected mitochondrial oxidative function, but also increased the role of its calcium-dependent regulation and the importance of these mechanisms in maintaining pro- and antioxidant homeostasis. In particular, Ca^{2+}-dependent mechanisms altered the oxidative function of mitochondria both at the I and II complexes of the electron transport chain of liver mitochondria. L-type calcium channel activity also contributed to increase pro-oxidant reactions and partially mediated the intensity of antioxidant mechanisms.

Regarding the difference in the effect of blocking calcium channels on the background of HFD (insulin resistance) and without it, it is known [22] that in patients with type II diabetes verapamil and similar selective blockers of slow calcium channels protect both cardiomyocytes and blood vessels in diabetes and related diseases, especially atherosclerosis and hypertension. It was shown [23, 24] that the mechanisms of calcium signaling depend on the presence of insulin resistance. Since the ROS generation is blocked by activating the PI3K-pathway, we can confirm the protective effect of CCB on cells during the development of inflammation [22, 25]. At the same time, in our study a negative effect on mitochondrial oxidative function was observed when CCB was used in the context of a high-fat diet. This was observed for both oxygen-dependent respiratory substrates and substrates that are products of lipid metabolism [26]. Furthermore, our results suggest that CCB may have a protective effect on the liver by reducing malondialdehyde levels [27, 28].

Thus, insulin resistance may alter the functioning of calcium signaling mechanisms and their regulatory influence on the functions of liver mitochondria both directly (due to changes in the oxidative function of hepatocyte mitochondria) and indirectly – due to effects on the cardiovascular system.

CONCLUSIONS

1. The oxidative function of liver mitochondria depends on the state of L-type calcium channels. Blockade of L-type calcium channels mediated a significant decrease in the level of V3/V4 in liver

mitochondria during oxidation of all substrates due to the inhibitory effect on the V3 and the stimulatory effect on the V4.

2. HFD-induced insulin resistance suppresses mitochondrial oxidative function during oxidation of both FAD- and NAD-dependent substrates. Under these conditions, blockade of L-type calcium channels stimulates V2 during oxidation of NAD-dependent but not FAD-dependent substrates, and also stimulates V3 and V4 during oxidation of the lipid substrate palmitoyl.

3. The effect of the blocker of L-type calcium channels completely or partially compensated the violations of the pro- and antioxidant status caused by using HFD. This indicates the involvement of these channels in the development of redox balance disorders in the liver during insulin resistance.

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БЛОКАДА КАЛЬЦІЄВИХ КАНАЛІВ L-ТИПУ ЗМІНЮЄ ФУНКЦІЮ МІТОХОНДРІЙ ПЕЧІНКИ У ЩУРІВ З ІНСУЛІНОРЕЗИСТЕНТНІСТЮ

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Мітохондрії є ключовими органелами підтримання енергетичного та оксидативного гомеостазу. Їх функція та механізми регуляції за фізіологічних умов і при інсулінорезистентності потребують поглибленого вивчення. Метою нашої роботи було вивчити вплив блокади кальцієвих каналів L-типу у щурів з інсулінорезистентністю на окиснювальну функцію мітохондрій печінки та зміни її оксидативного статусу. У щурів-самців лінії Вістар віком 6 міс моделювали інсулінорезистентність з утриманням на високожировій дієті впродовж 14 діб. Контролем слугували тварини, які перебували на стандартній дієті. Для блокади кальцієвих каналів L-типу вводили верапаміл інтраперитоніально у дозі 1 мг/кг. У гомогенаті печінки, вилученої у наркотизованих тварин через 3 год, визначали показники про- та антиоксидантної систем (активні продукти тіобарбітурової кислоти (ТБК-АП), відновлений глутатіон, каталаза, Cu-,Zn-супероксиддисмутаза). Функцію мітохондрій досліджували полярографічним методом за Chance з використанням різних метаболічних субстратів. Показано, що у інтактних щурів блокада кальцієвих каналів L-типу знижувала ефективність мітохондріального дихання (V3/V4) у печінці при окисненні всіх субстратів внаслідок гальмівного впливу на АДФ-стимульоване дихання (V3) і активуючого впливу – контрольоване дихання (V4), і не впливала на оксидативний статус печінки. У щурів з інсулінорезистентністю зменшувалось значення V3 при окисненні як НАД-, так і ФАД-залежних субстратів, виявлялись порушення оксидативного статусу та зростання антиоксидантного захисту. Проте у інсулінорезистентних щурів блокада кальцієвих каналів L-типу вірогідно стимулювала дихання у спокої (V2) при окисненні НАД-залежних субстратів, та V3 і V4 при окисненні ліпідного субстрату пальмітоїлу, пригнічувала співвідношення V3/V4 порівняно з контролем, водночас частково або повністю відновлювала порушення оксидативного статусу. Це може свідчити про залучення кальцієвих механізмів у порушення оксидативного статусу печінки та регуляцію енергетичного метаболізму в мітохондріях при інсулінорезистентності.

Ключові слова: інсулінорезистентність; мітохондрії; енергетичний метаболізм; редокс-баланс; кальцієві канали L-типу.

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