Imipramine prevents lipid accumulation in skeletal muscle at old age

Peer review status: No

Corresponding Author: Prof. Nataliya A Babenko,
Head of Department, Department of Physiology of Ontogenesis, Institute of Biology, Kharkov Karazin National University, 4, Svobody pl., 61077 - Ukraine

Submitting Author: Prof. Nataliya A Babenko,
Head of Department, Department of Physiology of Ontogenesis, Institute of Biology, Kharkov Karazin National University, 4, Svobody pl., 61077 - Ukraine

Other Authors: Mrs. Olga Tymofiychuk,
PhD student, Department of Physiology of Ontogenesis, Institute of Biology, Kharkov Karazin National University, 4, Svobody pl., Kharkov, 61077 - Ukraine

Article ID: WMC004423
Article Type: Research articles
Submitted on: 11-Oct-2013, 03:03:00 PM GMT Published on: 12-Oct-2013, 05:33:42 AM GMT
Article URL: http://www.webmedcentral.com/article_view/4423
Subject Categories: BIOCHEMISTRY
Keywords: Imipramine, Acid sphingomyelinase, Free fatty acids, Neutral lipids, Skeletal muscles, Aging
How to cite the article: Babenko NA, Tymofiychuk O. Imipramine prevents lipid accumulation in skeletal muscle at old age. WebmedCentral BIOCHEMISTRY 2013;4(10):WMC004423
Copyright: This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC-BY), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
Source(s) of Funding: This work was supported by the research program “Sphingomyelin cycle metabolites role in the development of cells resistance for physiological stimulus action during ageing” (State Registration No. 0111U010555).
Competing Interests: None
Imipramine prevents lipid accumulation in skeletal muscle at old age

Author(s): Babenko NA, Tymofiychuk O

Abstract

Acid sphingomyelinase (ASMase) deficiency abolishes palmitate- and saturated fat enriched diet-induced neutral lipid accumulation in liver cells and hyperglycemia. Niemann-Pick patients with ASMase deficit maintain low body weight. A reasonable assumption has been made that ASMase plays an important role in obesity. The lysosomal ASMase inhibitor tricyclic antidepressant imipramine effects on free fatty acids (FFA) and neutral lipids accumulation in muscles (soleus, gastrocnemius, extensor digitorum longus muscles, and diaphragm) have been investigated in aged rats. The 3- (adult) and 24-month-old (old) male Wistar rats were used in the experiments. Old animals were divided into 2 groups: control and imipramine-treated rats. Being administered, this drug was able to reduce triacylglycerol and diacylglycerol contents in all muscle tissues studied down to their levels in adult animals and to increase soleus sensitivity to the insulin action at old age. However, the drug injection to the 24-month-old rats did not change the FFA content in the extensor digitorum longus muscle. These results suggest that imipramine has tissue-dependent effect on FFA and can be an useful tool for improvement of neutral lipids levels and insulin resistance in skeletal muscle of old animals.

Introduction

Acid sphingomyelinase (ASMase) plays an important role in the regulation of sphingolipid homeostasis. ASMase hydrolyses sphingomyelin (SM) and generates ceramide, which is the player in numerous signaling pathways. Ceramide decreases the insulin-stimulated glucose and amino acids uptake by skeletal muscle [1, 2]. Ceramide suppresses IRS-1 phosphorylation in liver cells [3] and inhibits Ras activation, Glut-4 translocation, and Akt-1 phosphorylation in muscle cells [4, 5]. This mechanism contributes to the palmitate- or diet-induced insulin resistance [6, 7]. ASMase is overexpressed in adipose tissue of ob/ob mice [8] and in different tissues in the process of physiological aging [9, 10]. Recently, improvement of SM level and ceramide/SM ratio has been determined in liver, brain, heart and blood serum of old melipramin-treated rats [11]. The tricyclic antidepressants (imipramine, desipramine, amitriptyline, etc) are able to enter the lysosomes and to induce proteolytic degradation of ASMase, abolishing enzyme activity [12, 13]. Using mice deficient in ASMase ((asm−/−) it has been demonstrated that fat-enriched diet did not change significantly the weight of body and adipose tissue in contrast to asm+/+ animals. Niemann-Pick patients with ASMase deficit maintain very low body weight [14]. A reasonable assumption has been made that ASMase plays an important role in obesity.

In the present study, we investigated the link between the age-dependent elevation of ASMase activity and lipids accumulation in skeletal muscle and insulin resistance development in old animals. We demonstrated that the triacylglycerol (TAG), diacylglycerol (DAG) and free fatty acids (FFA) contents increased in skeletal muscle of old rats as compared with the adult ones. Lipids accumulation in muscle tissues was associated with the insulin resistance development in aged animals. Administration of functional inhibitor of ASMase, imipramine, to old rats nullified the age-dependent TAG and DAG increase in tissues and improved insulin sensitivity of skeletal muscles. The results obtained provide evidence that ASMase may play an important role in age-dependent disturbances of lipogenesis and insulin-stimulated glucose uptake by muscle tissues.

Methods

Animals

The 3- (adult) and 24-month-old (old) male Wistar rats were used in the experiments. They were kept at 24°C on a cycle of 12 h light/12 h darkness and had a free access to a standard chow diet and drinking water ad libitum. The experimental procedures were approved by the Institutional Animal Care and Use Committies at the Kharkov Karazin National University. The 24-month-old animals were divided into two groups: control (injected intra-muscularly with 0.9% NaCl for 14 days) and imipramine-treated (injected intra-muscularly with the drug (10 mg/kg body weight)}
daily for 14 days) rats. The animals were starved overnight prior to the experiment. Muscles (soleus, gastrocnemius, extensor digitorum longus muscles, diaphragm) were obtained 24 h after the last 0.9% NaCl or melipramin injection. The tissues were frozen in liquid nitrogen, stored at -80°C and further analyzed as described below.

**Extraction and Separation of Lipids**

The lipids were extracted (Bligh and Dyer (1959) [15]. The chloroform phase was collected and dried under N₂ at 37°C. The lipids were redissolved in chloroform/methanol (1:2, v/v) and applied on TLC plates. For lipids separation the solvent system: hexane/diethyl ether/acetic acid (36.5:12.5:1, v/v) was used. The appropriate standards were applied on each plate for quantification. The gel spots containing lipids were scraped and contents of lipids in chromatographic fractions were determined by the method of Marsh and Weinstain (1966) [16]. Content of protein in the samples was determined according to Lowry et al. (1951) [17].

**Glucose uptake**

The 2-dexy-D-[1,2-3H]glucose uptake was investigated in soleus muscle. Briefly, the muscle strips were incubated in an oxygenated (95% O₂/5% CO₂) Krebs/bicarbonate buffer (117 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, and 24.6 mM NaHCO₃) and 0.1% albumin (pH 7.5) for 60 min at 37°C. One muscle strip was stimulated with bovine insulin (100 nM) and the other was not. In 30 min the 2-dexy-D-[1,2-3H]glucose (0.5 µCi) was added to both strips for additional 10 min. Muscle strips washed in Krebs/bicarbonate buffer (pH 7.5) were digested in 1 N NaOH and heated for 10 min at 80°C. The glucose incorporated into tissues was then quantified by scintillation counting.

**Statistical analysis**

The data were analyzed by one-way analysis of variance (ANOVA) followed by post hoc Fisher’s protected least significant difference (Fisher PLSD) test. The results shown represent the means ± standard error of the mean (SEM) and deemed statistically significant when p< 0.05.

**Results and Discussion**

Pharmacological inhibition or genetic depletion of genes controlling sphingolipid metabolism prevents an excessive accumulation of TAG in the liver and hepatic steatosis development [18]. Chronic administration of inhibitor of sphingolipids synthesis myriocin to genetically obese ob/ob mice or wild animals with diet-induced obese decreased significantly the body weight, leptin mRNA expression in epididymal fat pads and plasma ceramide and sphingosine, and liver TAG contents [19]. Besides, the inhibition of ceramide synthesis with myriocin improves glucose tolerance and insulin sensitivity in liver and muscle. Treatment with myriocin of the rats with a lipid cocktail-induced insulin resistance reduced ceramide accumulation in skeletal muscle and improved glucose homeostasis [20]. Myriocin abolished the ceramide accumulation induced with palmitic acid or C₂- ceramide (D-erythro-N-acetylsphingosine) and reduced the insulin-stimulated glucose uptake and glycogen synthesis in "adult" hepatocytes [21]. These data clearly demonstrated the important role of newly synthesized ceramide in insulin resistance development. However, myriocin could not decrease ceramide content and improve insulin-induced glucose uptake and glycogen synthesis in liver cells of old rats up to the level in hepatocytes of adult animals [21].

Previously we reported that not only the myriocin but the inhibitor of ASMase imipramine could significantly reduce the ceramide accumulation in liver cells of old rats [22]. The long-term treatment of old rats with imipramine reduced the elevated ASMase activity in liver, blood serum and brain [11]. Taking into account the results obtained and the data on increased ASMase activity in liver cells and brain in the course of ageing [9, 10], reasonable assumption can be made that the ASMase plays an important role in ceramide accumulation in different tissues at old age.

In the study, we demonstrated that neutral lipids (Fig. 1A, B) and FFA (Fig. 1 C) contents increased in muscle tissues of 24-month-old rats as compared with 3-month-old animals. In addition, soleus sensitivity to the insulin action significantly decreased at old age. Basal and insulin-induced 2-dexy-D-[1,2-3H]glucose uptake in soleus muscle of 3-month-old rats was 788 ± 26 and 1680 ± 82 cpm/mg protein (P<0.05), respectively. While, basal and insulin-induced glucose uptake in muscle of old animals was 465 ± 18 and 508 ± 16 cpm/mg protein, respectively.

It is well documented that insulin resistance correlates highly with TAG content in skeletal muscle. However, TAG itself does not disrupt the insulin signaling in the target tissues. It is of interest, that DAG accumulation activated the protein kinase (PKC), phosphorylation of insulin receptor substrate-1 (IRS-1), which suppresses IRS-1 tyrosine phosphorylation and diminishes phosphatidylinositol 3-kinase (PI3K) activation and...
thus blocks the insulin signaling in the cells [23 - 27]. FFA accumulation in cells could cause not only to the TAG and DAG oversupply, but the overproduction of known antagonist of PI3K-dependent signaling pathways, ceramide. Culturing of the liver cells and diaphragm strips in the presence of palmitic acid increased significantly the contents of TAG, DAG, FFA and ceramide and decreased stimulation by insulin the \[^{3}H\]-D-glucose intake by the studied tissues [21, 28]. These findings suggest that neutral lipids as well as ceramide accumulation in the insulin-responsive tissues are sufficient for the inhibition of insulin-induced glucose uptake by the cells.

Imipramine administration to old rat significantly increased soleus sensitivity to insulin action and normalized the lipids contents in muscles. It has been shown that the drug increased the insulin-induced 2-deoxy-D-[1,2-\(^{3}H\)]glucose uptake by soleus strips of 24-month-old rats from 701 ± 15 in control to 1375 ± 31 cpm/mg protein (p<0.05) in the hormone-treated muscle. Moreover at old age, the imipramine reduced elevated DAG, TAG and FFA contents in diaphragm (Fig. 1 A, B, C), gastrocnemius (Fig. 2 A, B, C), soleus (Fig. 3 A, B, C) and neutral lipids in the extensor digitorum longus muscles (Fig. 4 A, B). Injection of the drug to the 24-month-old rats did not change the FFA content in the extensor digitorum longus muscle. Previously we found that the imipramine decreased significantly the ceramide content and acid SMase activity and increased the SM levels in the diaphragm, gastrocnemius, soleus and in the extensor digitorum longus muscles (in press). Taking into account these results, it can be supposed that at old age the age-dependent disturbances of sphingolipid turnover play an important role in neutral lipids and FFA accumulation in muscle tissues and insulin resistance development.

Antidepressant drugs-induced weight gain is a well known problem in the treatment of psychiatric disorders. Weight gain side-effects of antidepressant drugs administration are strongly associated with increased lipid biosynthesis and dyslipidemia. By using the imipramine, amitriptyline and some other antidepressants, a pronounced activation of sterol regulatory element-binding protein (SREBP) transcription factor and up-regulation of downstream genes involved in cholesterol- and fatty acid biosynthesis in cultured glial and liver cells have been found [29, 30]. However, the imipramine decreased the weight of mice after a four-week-long treatment as compared to the controls [31] and had no effect on 24-month-old rat body weights after a two-week-long treatment. The body weight of control and imipramine-treated rats were 436 ± 14.4 and 405 ± 12.1 g, respectively. It has been also demonstrated that imipramine abolished neutral lipids and FFA accumulation in muscle tissues of old animals (Fig. 1 - 4). Previously we reported that two-week-long imipramine administration to 24-month-old rats increased significantly the phosphatidylincholine and phosphatidylyethanolamine contents in the blood serum, diaphragm, gastrocnemius and soleus muscles [32]. The tricyclic antidepressants administration resulted in decreased degradation and increased glycerolphospholipids synthesis in cells [33, 34]. It could not be excluded that the FFA newly synthesized in the liver can be used for glycerolphospholipids synthesis in the muscles rather than for DAG and TAG synthesis. However, the mechanism of imipramine targeting of FFA to glycerolphospholipids synthesis requires further investigations.

Conclusions

The results obtained demonstrated that neutral lipids and FFA accumulation in skeletal muscle tissues at old age was associated with the insulin resistance development. 2-week-long imipramine treatment of old animals abolished the age-dependent disturbances of lipid metabolism and improved tissues sensitivity to insulin action. Taking into account that imipramine is a strong acid SMase inhibitor and improves both lipids contents and muscles sensitivity to insulin, a reasonable assumption can be made that up-regulation of acid SMase plays an important role in the insulin resistance development at old age.

Authors Contributions

NB conceived the study and participated in its design, coordination, and manuscript preparation. OT participated in data collection and performed the statistical analysis.

References

3. Herschkovitz A, Liu YF, Ilan E, Ronen D,


Effect of imipramine on the lipids contents in the diaphragm of 24-month-old rats. DAG (A), TAG (B) and FFA (C).

1-3 month old rats; 2- 24 month-old rats; 3- 24 month-old rats, control (0.9 % NaCl); 4 - 24-month-old rats, imipramine. * 0.05 (24-month-old rats vs 3-month-old animals, **; 0.05 (24-month-old drug-treated rats vs 24-month-old control animals).

Illustrations

Illustration 1

Figure 1
Effect of imipramine on the lipids contents in the gastrocnemius muscle of 24-month old rats. DAG (A), TAG (B) and FFA (C). 1-3 month-old rats; 2-24 month-old rats; 3-24 month-old rats, control (0.9 % NaCl); 4-24 month-old rats, imipramine. * 0.05 (24-month old rats vs 3-month old animals, **; 0.05 (24-month-old drug-treated rats vs 24-month-old control animals)

Illustration 2

Figure 2

[Bar graphs showing the effect of imipramine on DAG (A), TAG (B), and FFA (C) in gastrocnemius muscle of 24-month old rats.]

- A: DAG levels with different shading to represent groups 1-4.
- B: TAG levels with different shading to represent groups 1-4.
- C: FFA levels with different shading to represent groups 1-4.
Effect of imipramine on the lipids contents in the soleus muscle of 24-month old rats. DAG (A), TAG (B) and FFA (C). 1-3 month-old rats; 2-24 month-old rats; 3-24 month-old rats, control (0.9 % NaCl); 4-24 month-old rats, imipramine. * 0.05 (24-month-old rats vs 3-month-old animals, ** 0.05 (24-month-old drug-treated rats vs 24-month-old control animals).
Effect of imipramine on the lipids contents in the extensor digitorum longus of 24-month old rats. DAG (A), TAG (B) and FFA (C). 1-3 month-old rats; 2-24 month-old rats; 3-24 month-old rats, control (0.9 % NaCl); 4-24 month old rats, imipramine. * 0.05 (24-month-old rats vs 3-month-old animals, ** 0.05 (24-month-old drug-treated rats vs 24-month-old control animals).