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Mechanisms for glyproline protection in hypercholesterolemia

Nikolay F. Myasoedov[a], Ludmila A. Lyapina[b,*], Marina E. Grigorjeva[b], Tamara Y. Obergan[b], Tatyana A. Shubina[b], Ludmila A. Andreeva[a]

[a] Institute of Molecular Genetics, Russian Academy of Sciences, Moscow, Russia
[b] Biology Department, M. V. Lomonosov Moscow State University, Moscow, Russia

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ABSTRACT

Comparative analysis of the hypocholesterolemic and antithrombotic action of small regulatory glyproline peptides (Pro-Gly-Pro, Arg-Pro-Gly-Pro and Pro-Gly-Pro-Leu) was performed on an experimental hypercholesterolemia model of rats. Repeated intranasal introduction of glyproline peptides to fat-diet-fed animals led to more active functioning of the anticoagulation system (the anticoagulant and fibrinolytic properties of the plasma increased and platelet aggregation decreased) and to normalization of the total cholesterol level as a parameter of lipid metabolism. The largest anticoagulant and hypocholesterolemic effect was detected for the Pro-Gly-Pro-Leu peptide. Hypothetical mechanisms of antithrombotic and hypocholesterolemic effects of glyproline peptides are presented.

1. Introduction

The liver plays a key role in fat metabolism for the synthesis and consecutive release of cholesterol from hepatocytes into the blood and bile from food-supplied lipids [1]. Cholesterol, phospholipids, triacylglycerides and unesterified fatty acids are the principal blood lipids forming parts of cell membranes. The majority of the blood lipids are transported in lipoprotein particles whose sizes are regulated by extracellular lipases. Partial hydrolysis of triglycerides is accompanied by an increased concentration of apoproteins, which are recognized by more than ten different lipoprotein receptors [2].

Hyperlipemia is one of the main symptoms of fat metabolism disorders. Alimentary hyperlipemia is observed two to three hours after lipid uptake, and the lipid level returns to a normal level after nine hours. A decreased level of heparin, which activates blood lipoprotein lipase as well as during a splenectomy and a blockade of mononuclear phagocytes, is accompanied by an increased duration and higher level of hyperlipemia. Similar effects are produced by sodium chloride and fat acids. Retentional hyperlipemia is caused by changes in the ratio of blood protein fractions. Transport hyperlipemia is observed in the case of a glycogen shortage in the liver (starvation, diabetes mellitus) as well as at elevated levels of certain hormones. Lipid accumulation outside of adipose tissue is designated as fat infiltration. A combination of infiltration and failure of adipocyte protoplasm is defined as fat dystrophy, which is caused by decreased activity of hydrolytic and oxidative enzymes induced by viral infection, avitaminosis or arsenic poisoning [3]. Prolonged emotional stress leads to mobilization of fat and to weight loss. Similar effects are observed after sympathetic nerve stimulation, whereas activation of parasympathetic nerves is accompanied by fat accumulation [4].

Disturbances of fat metabolism can be induced by many different reasons; among them are changes in digestion and absorption of lipids in the small intestine, changes in lipid transportation, abnormalities of lipid deposition, malfunctioning of the liver as well as changes in nervous and hormonal regulation of fat metabolism [4]. It has been established [2] that increased uptake of cholesterol can also induce fat metabolism disorders. This can be due to cholesterol-induced changes in lipoproteins resulting in hypercholesterolemia (dyslipidemia) and in the development of atherosclerosis and atherothrombosis [5,6]. Under these conditions, cholesterol is accumulated in certain regions of damaged vascular walls, causing atherosclerotic plaque formation [7].

It has been established that endothelium damaged by oxidized cholesterol complexes of low-density lipoproteins (LDL) and

Abbreviations: HFD, hypercholesterol fat diet; TC, total cholesterol; HDL, high-density lipoproteins; LDL, low-density lipoproteins; VLDL, very low-density lipoproteins; TFA, total fibrinolytic activity; NF, non-enzymatic fibrinolytic activity; EF, enzymatic fibrinolytic activity; t-PA, tissue plasminogen activator; APTT, activated partial thromboplastin time; NO, nitric oxide; PRP, platelet-rich plasma; PPP, platelet-poor plasma; PGP, Pro-Gly-Pro; RPGP, Arg-Pro-Gly-Pro; PGPL, Pro-Gly-Pro-Leu.

* Corresponding author at. Biology Department, M. V. Lomonosov Moscow State University, Laboratory of Blood Protective Systems, 1/12, Moscow 119991, Russia.
E-mail addresses: anfm@img.ras.ru (N.F. Myasoedov), lyapinal@mail.ru (L.A. Lyapina), mgrigorjeva@mail.ru (M.E. Grigorjeva), tobergan@mail.ru (T.Y. Obergan), shubina.74@mail.ru (T.A. Shubina), landr@img.ras.ru (L.A. Andreeva).

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very low-density lipoproteins (VLDL) loses its ability to produce endogenous vasodilators. This can be accompanied by an increased tendency of vessel spasms, which are especially dangerous in the case of coronary arteries [7]. Triglycerides utilized for transportation and fat storage can be used for estimating the risk of cardiovascular diseases. Disturbances of cholesterol metabolism can also be accompanied by cholelithiasis, lipid nephrosis, age corneal opacity, skin and bone xanthomatosis and other diseases. Physiologically, normal cholesterol levels in the blood of adults are in the range of 3.5–5.6 mmol/l [8]. Cholesterol concentration is increased upon consumption of cholesterol-rich foods (egg yolk, fried brains, liver, butter, etc.). However, this alimentary hypercholesterolemia is temporary because cholesterol induces liberation of heparin from mast cells. Heparin activates lipoprotein lipase, decreasing the total level of cholesterol and LDL cholesterol in blood [9].

Glucocorticoids induce fat mobilization in adipose tissue and inhibit lipogenesis. However, these effects are undercounted by glucocorticoid-induced stimulation of insulin secretion and hyperlipidemia. Insulin blocks the liberation of fat from depots and enhances glycogen accumulation in the liver and the lipid conversion of carbohydrates. Insulin increases appetite by decreasing the level of glucose in the blood. All of these effects lead to lipid accumulation in adipose tissue and obesity [9]. Insulin and prolactin activate the pentose-phosphate pathway and glycolysis thus increases the lipid conversion of carbohydrates and the accumulation of fat in the corresponding depots [10,11].

Hypercholesterolemia enhances the formation of thrombotic microparticles, increases blood coagulation and activates receptors of LDL cholesterol [12]. Disturbances of fat metabolism often lead to atherothrombosis. Therefore, antithrombotic therapy is important for patients with disturbed fat metabolism [3,13,14]. All of the above-mentioned facts indicate the urgency of the search for antilipemic agents.

The natural peptide hormone leptin [15], produced by adipose tissue, is involved in the maintenance of normal blood glucose levels. Leptin controls the feeling of hunger through the hypothalamus. The accumulation of excess fat induces an increased level of leptin and decreased appetite, thus providing for fat utilization. Conversely, decreased leptin levels increase appetite and contribute to extra fat accumulation [9].

By normalizing arterial blood pressure, the level of triglycerides and the balance of lipoproteins in the blood, certain amino acids (proline, lysine, arginine, valine, glutamine, leucine and others) can prevent the deposition of LDL cholesterol and reduce the risk of certain diseases, including diabetes, atherosclerosis, and atherothrombosis [13,16].

Arginine is a precursor of nitric oxide (NO), which is formed by NO-synthases. The regulation of NO-synthase proceeds according to a negative feedback mechanism. NO is involved in a number of physiological functions, including vasodilatation, neurotransmission, inhibition of platelet aggregation, regulation of smooth muscle tone, etc [17]. Thus, arginine inhibits blood coagulation and improves the rheological properties of blood, activates fibrinolysis, elevates antiplatelet activity, normalizes blood sugar levels [5,18] and neutralizes free radical accumulation. By these means, arginine decreases the risk of cardiovascular diseases, atherosclerosis and diabetes mellitus [19].

Leucine is one of the essential amino acids. Its absence or deficiency can cause metabolic disturbances or problems with growth and/or development. Leucine is used in the therapies of liver diseases, muscular dystrophy and of some nervous system diseases. Moreover, leucine participates in the maintenance of nitrogen balance in the body and in protein and carbohydrate metabolism and is required for the formation and growth of muscle tissue. Additionally, it decreases sugar levels in the blood [20]. Leucine stimulates the glucose-alanine cycle, thus stabilizing the glucose level and maintaining muscle mass under the conditions of a low calorie diet. This amino acid is found in hazel nuts, beans, soy flour, brown rice, egg whites, meat (beef fillet, chicken breast, and salmon) and whole wheat. Reduced intake of carbohydrates considerably decreases the levels of insulin sending the fat-retention signal to adipose tissue fibers. Experiments by Dr. Matthias Bluher at Harvard Medical School indicate that deprivation of the adipose receptor of insulin provides almost complete protection from obesity. Experimental rodents with defects of this receptor gained no excess weight obtained from any type of food. Thus, it is clear that insulin plays a critical important role in fat accumulation processes and that this is independent of the number of calories consumed [21].

The essential amino acid valine is involved in the growth and synthesis of the body's tissues, is necessary for normal nitrogen metabolism and belongs to the branch-chained amino acid family. Recently published data indicate that valine and leucine increase the level of high-density lipoprotein (HDL) cholesterol and, by these means, reduce the risk of cardiovascular diseases, especially in patients with hypercholesterolemia and atherosclerosis. It is presumed that these amino acids, when used as food additives, can prevent the progress of hyperlipidemia [20].

Proteolysis of collagen and relative proteins is accompanied by accumulation of the short peptides Pro-Gly-Pro, Pro-Gly, Pro-Gly-Pro-Arg and Pro-Gly-Arg. These peptides ensure normal continuous functioning of the insular and anticoagulant blood systems in an organism [22–25].

Given that hyperglycemia causes fat accumulation and that the above-mentioned glyprolines possess a hypoglycemic effect, one may presume that these small peptides, especially with the addition of leucine, valine or arginine, will be able to participate in not only carbohydrate but also fat metabolism. Lipid metabolism corrections are conducted by diet control and physical exercise, both resulting in overweight reduction. It has been established that regular exercise reduces the risk of cardiovascular conditions, brain diseases and other disorders by decreasing the level of triglycerides and raising the level of HDL cholesterol. Both of these factors produce a positive effect on fat metabolism [26]. Statins, the drugs presently used for decreasingcholesterol levels, have adverse effects that are harmful for the body and can cause pancreatitis, cholestatic jaundice, hepatitis, biliary colic, among others [27].

The development of drugs that are able to activate the functions of the anticoagulant, fibrinolytic and antiplatelet stages of the anticoagulation system and to influence the subtle mechanisms of thrombosis led to the elaboration of therapies using inhibitors of platelet glycoproteins IIb-IIIa. The use of these peptide preparations is limited however by their short-term activity. Currently, the struggle with atherothrombosis is considered unsatisfactory [6].

This study, utilizing an experimental hypercholesterolemia model in rats, presents a comparative analysis of the hypocholesterolemic and antithrombotic actions of small regulatory glyproline peptides, including Pro-Gly-Pro, Arg-Pro-Gly-Pro and Pro-Gly-Pro-Leu.We also present a hypothetical mechanism of action of these peptides.

2. Methods

2.1. Peptides and peptide solution preparations

The glyproline-based peptides Pro-Gly-Pro (PGP), Arg-Pro-Gly-Pro (RPGP), and Pro-Gly-Pro-Leu (PGPL) were synthesized and obtained from the Institute of Molecular Genetics of the Russian Academy of Sciences (Moscow, Russia). Samples of peptides were dissolved in 0.85% saline (as vehicle) daily and were introduced