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Research Article

Hepatoprotective effect of opioid peptides in stress

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Abstract

Introduction: Influence of the endogenous opioid system on the liver has not been studied enough. To understand the damaging effects of stress on the liver and the hepatoprotective effects of opioids, a study was performed on stress-resistant and stress-susceptible animals.

Materials and methods: The investigation was performed on 725 Wistar male-rats. Various types of stress were modeled: acute immobilization stress of various duration (3, 6 and 12 hours), chronic stress of limited mobility, swimming stress and traumatic stress (resection of 70% of the liver). Agonists of various types of opioid receptors in equimolar doses were injected to stressed animals at equimolar doses: DAGO – a mu-receptor agonist – at a dose of 6.3 mcg/kg, DSLET – a delta-receptor agonist – at a dose of 10 mcg/kg, and kappa receptor agonist dynorphin A (1-13) – at a dose of 20.1 mcg/kg.

Results and discussion: The stress-limiting action of the studied opioids is characterized by the reduced hepatocyte dystrophy, microcirculation correction, a decreased concentration of lipid peroxidation metabolites, a suppressed cytolytic syndrome, a stimulated synthetic ability of the liver, and is more pronounced in stress- susceptible animals. The greatest stress-protective effect is shown after administering dynorphin A (1-13) in immobilization stress, and DAGO – in swimming stress. Dynorphin A (1-13) and DAGO manifested the most pronounced effect on the liver regeneration after resection. A preliminary stress simulation accelerates liver regeneration at the initial stage after resection.

Conclusion: The hepatoprotective effect of opioids in stress depends on the typological peculiarities of animals. The results obtained offer a challenge of synthesizing new hepatoprotectors.

Keywords

stress, liver, liver resection, opioid peptides, hepatoprotectors.

Introduction

Stress causes a disturbance in the homeostasis of the body, leading to changes in many organs and systems. Pertsov et al. (2015) determined the specific features of the response of individuals to the action of the same stress stimuli. Based on numerous studies, the idea of individuals being stress-resistant and stress-susceptible was formulated. It is generally recognized that the leading mechanism that determines the different resistance of animals and

people to stress is the functioning of stress-mediating and stress-limiting systems (Pshennikova 2003).

One of the target organs of stress is the liver. It was established that the development of stress was accompanied by morphological and functional changes in the liver tissue (Lyashev and Serikov 2016, Serikov and Lyashev 2015). The development of stress is accompanied by the inhibition of microsomal reductases, a disruption of hydroxylation, and disturbed synthesis of coenzymes.

One of the forms of emergency exposure is postoperative stress, when a patient, along with traumatic tissue damage, has a stress response due to a combination of factors. However, carrying out massive liver resections is often accompanied by the development of a severe complication – post-resection liver failure (Mullen et al. 2007). Reducing the severity of post-resection complications is achieved by stimulating the recovery of the volume of functioning tissue, since it is known that liver tissue has a high regenerative activity (Mangnall et al. 2003).

A promising area of prevention and correction of stress-induced damage is the use of synthetic analogues of natural opioid peptides (OP). Currently, the endogenous opioid system (EOS) is considered as the leading component of the anti-stress system of the body (Drolet et al. 2001, Lishmanov and Maslov 1994). The existence of at least 4 types of opioid receptors (OR) was established: mu, delta, kappa, ORLI (opioid like receptor), as well as subtypes: mu_1, mu_2-, mu_3-, $delta_1$- and $delta_2$-, $kappa_{1alfa}$-, $kappa_{1betta}$-, $kappa_2$-, $kappa_3$-), for which selective agonists were synthesized (Granier et al. 2012, Manglik et al. 2012, Thompson et al. 2012, Wu et al. 2012). OPs having both a stress-limiting effect and a stimulating effect on regeneration make it possible to use them in liver resection to accelerate regeneration.

The purpose of this investigation is to explain the mechanisms of the hepatoprotective action of endogenous opioid system (EOS) components during stress.

Materials and methods

Experimental studies were conducted on 725 Wistar male rats weighing 180–250 g each. The investigation was performed in compliance with the European Convention for the Protection of Vertebrate Animals Used for Experiments or for Other Scientific Purposes [Directive 2010/63/EU]. All experimental studies were approved by the Ethics Committee of Kursk State Medical University, Ministry of Healthcare of the Russian Federation (protocol No1 of 28.05.2013). Vivisection was carried out in accordance with the ethical principles of treating laboratory animals and in compliance with the requirements of the "International recommendations for the conduct of biomedical research using animals" (1985).

In preliminary experiments, stress-resistant and stress-susceptible individuals were singled out from among the Wistar rat population in the open field test (Koplik 2002). The model of acute immobilization stress: fixation of an animal on its back on the laboratory table for 3, 6 or 12 hours. A two-hour swimming stress was modeled by placing the animal into a tank filled with water at t=22 °C. The level of liquid was such that the animal could keep on the surface only by swimming.

Animals were removed from the experiment after 39 hours, on the 4th and 7th days after completion of the stress simulation. The choice of these terms was based on the literature data that the period of 39 hours after stress corresponds to the end of the anxiety stage (pathological changes in the internal organs are most pronounced), the 4th day after the stress modeling is the beginning of the resistance stage; the 7th day – the maximum development of compensatory processes in the body (Vyborova et al. 2005).

To simulate chronic stress, long-term mobility restrictions were applied: for 12 days every day, from 9.00 am to 3 pm, the animals were placed into small-sized cells that restricted their mobility, without access to food and water (Neporada and Leont`eva 2003). The animals were removed from the experience on the 12th day.

The intensity of lipid peroxidation (LPO) was assessed by the concentration of the metabolites of these reactions in blood plasma and in the liver tissue homogenate: acylhydroperoxides (AHPs) and malondialdehyde (MDA), as well as by the activity of antioxidant enzymes: superoxide dismutase (SOD) and catalase, which were assessed by traditional methods (Alekseev et al. 2013, Galaktionova et al. 1998).

The concentrations of total cholesterol (TC), triglycerides (TG), the content of non-esterified fatty acids (NEFA), very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL) in plasma were determined by enzymatic colorimetric methods.

The activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), the concentrations of total protein, albumin, glucose, bilirubin in blood plasma were determined by traditional methods (Bais and Philcox 1994, Lorentz et al. 1993, Menshikova 1987).

For morphological studies, paraffin blocks were made from liver fragments of the animals, using a standard technique. The slides were stained by hematoxylin and eosin. The following indicators were determined planimetrically: the percentage of normal and dystrophically-changed hepatocytes relative to the total number of cells, the degree of dystrophic changes in the cytoplasm, the number of binuclear hepatocytes, the relative number of nuclei with one nucleolus, the diameter of nuclei of hepatocytes, and the specific volume of intrahepatic sinusoids.

Partial hepatectomy (PHE) was performed according to the method described by Higgins G.M. and Anderson R.M. (Higgins 1931). The animals were removed from the experiment on the 1st, 3rd, and 7th days of the experiment. The total mass of the right and caudate lobes was determined, and the following indicators were calculated: 1) a relative increase in the mass of the right and caudate lobes against the mass of these lobes after resection in

percent; 2) the relative mass of the liver (the ratio of the mass of the liver on the day of the study to the initial mass of the liver) in percent.

A median laparotomy was performed on the sham-operated rats, the liver was partially taken out of the abdominal cavity and then returned back. The laparotomy was sutured closed layer-by-layer. The animals were removed from the experiment within the same time periods as after PHE.

In the study, synthetic preparations of selective agonists of individual OR classes:

DAGO is a selective mu-OR agonist (Sigma-Aldrich, USA). Structure: H-Tyr-D-Ala-Gly-N-methyl-Phe-Gly-ol.

DSLET is a selective delta-OR agonist (Vector+, Novosibirsk, Russian Federation). Structure: H-Tyr-D-Ser-Gly-Phe-Leu-Thr-OH.

Dynorphin A (1-13) is a selective kappa-OR agonist (Vector+, Novosibirsk, Russian Federation). Structure: H-Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-OH acetate salt.

The lack of a selective ORLI agonist is explained by the fact that peptide selective agonists of this OR type have not yet been established (Pasternak 2011).

The studied OPs were used in equimolar doses: DAGO – 6.3 μg per kg of body weight, DSLET – 10.0 μg per kg of body weight, and dynorphin A (1-13) – 20.1 μg per kg of body weight. These doses were chosen on the basis of the previous experiments by the authors and literature data on the high activity of OP in these doses (Lyashev 2002).

The drugs used were dissolved in physiological solution and administered intraperitoneally to rats in a volume of 0.2 ml daily for 5 days after modeling an acute immobilization or swimming stress, and also intraperitoneally in a volume of 0.2 ml daily for 7 days from the 1st to the 7th days of modeling chronic stress of limited mobility. To study the effect of OP on liver regeneration after PHE, the studied peptides were administered intraperitoneally to rats in a volume of 0.2 ml daily for 5 days after surgery. The control animals were similarly injected with physiological solution.

The data obtained as a result of the research were processed statistically, using parametric and non-parametric methods. The significance of differences in the mean values in the case of a normal distribution in two samples was evaluated by Student's t-test, in the case of an abnormal distribution by Wilcoxon test. In order to identify hidden factors affecting statistical correlations, a factor analysis was carried out using the method of basic components. Statistical processing was performed using MS Excel and Statistica 10 software.

Results and discussion

Simulation of a 3-hour immobilization stress increased in the content of intermediate and final LPO metabolites in plasma only 39 hours after the end of exposure: the concentration of MDA increased 2.5 times ($p<0.001$), and of AHP – 2.6 times ($p<0.001$). There was an increase in SOD activity by 30.1% ($p<0.01$), while the activity of catalase did not change significantly ($p>0.05$).

The injection of selective OR agonists brought about an antioxidant effect in the stressed rats. Catalase activity increased at all periods of the experiment compared with the control group ($p<0.001$). After 39 hours, the concentration of MDA was significantly lower than that of the control group and, starting from the 4th day, it did not differ from that in the intact animals.

In the liver tissue, there was an increase in the content of MDA and AHP – by 75.3% and 60.3%, respectively, 39 hours after the 3-hour immobilization ($p<0.001$). There was also an increase in the activity of the both studied antioxidant enzymes – SOD and catalase – by 41.6% and 27.0%, respectively ($p<0.001$). After 4 days, there remained a higher concentration of AHP in the liver tissue (by 34.9%, $p<0.001$) compared with that of the intact animals (Table 1).

The use of OP had an inhibitory effect on LPO in the liver tissue of the stressed rats, which was manifested by a decrease in the concentration of MDA, but not of AHP (when injecting dynorphin A (1-13) – by 17.7%, DAGO – by 15.0%, DSLET – by 14.6% ($p<0.001$)) as early as 39 hours after stress modeling. When using all OPs, an increase in catalase activity in the liver tissue was found 39 hours after the exposure. An increase in SOD activity was established only with the use of DSLET ($p<0.001$) for all the periods of the experiment.

In the animals exposed to a 6-hour immobilization stress, there was an increase in the concentration of AHP in blood plasma – by 2.75 times ($p<0.001$) and of MDA – by 2.66 times ($p<0.001$) after 39 hours (Table 2). On the 4th day, these disorders persisted. The changes in the activity of antioxidant enzymes were multidirectional: catalase activity decreased by 22.4% ($p<0.001$), while SOD activity, on the contrary, increased by 11.5% ($p<0.05$). Seven days after a 6-hour stress, the indicators did not significantly differ from those of the intact animals.

The use of OP reduced a degree of LPO activation and prevented a decrease in catalase activity. DSLET had the most pronounced stress-limiting effect, which was manifested by a decrease in the concentration of LPO metabolites, an increase in catalase activity ($p<0.001$) compared with those in the control group throughout all the periods of the experiment.

In liver tissue, a 6-hour stress was accompanied 39 hours after by an increase in the content of MDA by 2.95 times ($p<0.001$) and of AHP – by 2.57 times ($p<0.001$). The increased concentration of LPO metabolites remained on the 4th and 7th days after immobilization (Table 2). There was an increase in catalase activity was noted: by 21.8% ($p<0.05$) and 20.4 % ($p<0.01$), on the 4th and 7th days, respectively. On the contrary, SOD activity decreased by 22.9% ($p<0.01$) on the 7th day, compared to that of the intact animals. The use of OP suppressed the activation of LPO and increased the activity of enzymes, especially SOD. Kappa-OR agonist dynorphin A (1-13)

Table 1. Effect of Opioid Peptides on the Concentration of Lipid Peroxidation Metabolites and the Activity of Antioxidant Enzymes in the Liver Tissue of Rats Exposed to 3-hour Immobilization (M±m, n=8).

Group \ Indicator	Period after stress simulation	Content of malonic dialdehyde, mcmol/g of tissue protein	Content of acyhydro-peroxides, su	Catalase activity, mcat/g of tissue protein	Superoxidis-mutase activity, su
Intact		17.4±0.4	6.3±0.3	21.1±0.9	23.1±1.1
Control (stressed)	39 hours	30.5±1.3^{xxx}	10.1±0.4^{xxx}	26.8±0.6^{xxx}	32.7±0.7^{xxx}
	4 days	17.0±0.3	8.5±0.2^{xxx}	21.3±0.9	25.0±0.8
	7 days	18.0±0.4	6.1±0.3	20.7±1.2	23.9±0.9
Stress + dynorphin A (1-13) at a dose of 20.1 mcg/kg	39 hours	25.1±0.9***	9.0±0.4	30.1±0.5***	32.2±0.8
	4 days	16.6±0.3	5.8±0.3***	21.2±0.7	23.4±0.7
	7 days	16.5±0.4*	5.8±0.2	24.8±1.1*	20.5±1.4*
Stress+ DAGO at a dose of 6.3 mcg/kg	39 hours	25.6±0.3***	8.0±0.2	28.7±0.5*	33.7±0.3
	4 days	15.9±0.4	7.5±0.5	17.2±0.4**	17.5±0.5***
	7 days	13.8±0.4***	5.5±0.3	32.6±0.5***	36.4±1.0***
Stress + DSLET at a dose of 10.0 mcg/kg	39 hours	25.7±0.3***	8.4±0.3	29.8±0.6**	35.0±0.6***
	4 days	15.9±0.4	6.2±0.4**	32.7±0.6***	36.9±1.0***
	7 days	15.4±0.6**	6.3±0.4	27.2±0.3***	35.4±0.4***

Note: ^{x} – p<0.05, ^{xx} – p<0.01, ^{xxx} – p<0.001 compared with intact animals. * – p<0.05, ** – p<0.01, *** – p<0.001 compared with control animals.

Table 2. The Effect of Opioid Peptides on the Content of Lipid Peroxidation Metabolites and the Activity of Antioxidant Enzymes in the Liver Tissue of Rats Exposed to 6-hour Immobilization (M±m, n=8).

Group \ Indicator	Period after stress simulation	Content of malonic dialdehyde, mcmol/g of tissue protein	Content of Acylhydro-peroxides, su	Catalase activity, mcat/g of tissue protein	Superoxi-dismutase activity, su
Intact		17.4±0.4	6.3±0.3	21.1±0.9	23.1±1.0
Control (stressed)	39 hours	51.3±1.2^{xxx}	16.2±0.7^{xxx}	23.3±1.2	23.7±0.8
	4 days	41.8±0.9^{xxx}	9.7±0.5^{xxx}	25.7±1.1^{x}	21.9±1.1
	7 days	32.8±0.7^{xxx}	10.6±0.4^{xxx}	25.4±0.8^{xx}	17.8±0.7^{xx}
Stress + dynorphin A (1-13) at a dose of 20.1 mcg/kg	39 hours	27.6±0.4***	9.3±0.6***	19.7±2.4	35.4±1.2***
	4 days	25.4±0.4***	6.3±0.3***	33.5±0.8***	39.7±0.8***
	7 days	17.5±0.3***	6.2±0.3***	21.6±1.9	46.3±0.6***
Stress+ DAGO at a dose of 6.3 mcg/kg	39 hours	43.2±0.5***	13.9±0.9*	23.5±0.8	20.6±1.0*
	4 days	37.8±0.5***	8.7±0.3	23.7±1.2	23.8±0.9
	7 days	25.6±0.4***	8.0±1.0*	24.5±0.6	24.3±1.2**
Stress + DSLET at a dose of 10.0 mcg/kg	39 hours	36.1±0.6***	10.1±0.7***	25.9±0.5	25.6±0.8
	4 days	27.5±0.5***	9.1±0.6	33.2±0.7***	38.4±1.2***
	7 days	15.9±0.9***	6.0±0.5***	20.4±1.4	35.8±1.1***

Note: ^{x} – p<0.05, ^{xx} – p<0.01, ^{xxx} – p<0.001 compared with intact animals. * – p<0.05, ** – p<0.01, *** – p<0.001 compared with control animals.

had the most pronounced effect, which was manifested by a normalized content of AHP and a significant decrease in the concentration of MDA as early as on the 4th day of the experiment. The peptide caused the most pronounced increase in SOD activity at all the periods of the experiment: after 39 hours – by 49.4%, on the 4th day – by 81.3%, and on the 7th day – by 2.6 times (p <0.001). Catalase activity was 30.4% (p<0.001) higher compared with that in the control group only on the 4th day. Under the influence of DAGO, the concentration of MDA decreased by 15.4% (p<0.01), and of DSLET – by 29.6% (p<0.001), of AHP – by 14.2% (p<0.05) and by 37.7% (p<0.001) after 39 hours, respectively, and remained statistically significantly lower compared with the control group. The use of DAGO did not affect the activity of catalase, whereas the peptide had an inhibitory effect on SOD activity 39 hours after exposure (by 10.8%, (p<0.05)), and a stimulating effect – by 36.5% (p<0.01) on the 7th day. The use of DSLET had a stimulating effect on catalase activity on the 4th day of the experiment, and on SOD – on the 4th and 7th days.

After a 12-hour immobilization, accumulation of lipid peroxidation metabolites in blood plasma of the stressed rats was observed throughout all periods of the experi-

ment. Seven days after the 12-hour immobilization, the concentrations of both MDA and AHP exceeded similar values in the intact animals (MDA – by 68.0%, $p<0.001$; AHP – by 42.9%, $p<0.05$). There was a decrease in catalase activity on the 7th day, but not of SOD. The use of all the studied OPs had an antioxidant effect in rats that exposed to a 12-hour immobilization stress. The most pronounced effect was shown by a selective delta-OR agonist DSLET. By the 7th day, the content of LPO metabolites did not differ from that in the intact animals and was significantly lower than that in the control group. The activity of antioxidant enzymes was significantly higher ($p<0.001$) compared with the control at all periods of the experiment.

In the liver tissue, the simulation of 12-hour stress was accompanied by the accumulation of MDA and AHP. SOD activity was reduced at all periods of the experiment, and catalase did not change significantly. The use of OP had a pronounced antioxidant effect. So, with the injection of DSLET, the concentration of MDA after 39 hours was 36.3% ($p<0.001$), and of AHP – by 52.3% ($p<0.001$) lower than those of the control animals. The activity of SOD and catalase significantly increased throughout all periods of the experiment ($p<0.001$). On the 7th day, the content of LPO metabolites did not differ significantly from that in the intact rats. The administration of dynorphin A (1-13) resulted in a decrease in the content of LPO metabolites in the liver tissue of rats throughout all periods of the experiment. SOD activity was higher than that in the control group at all stages of the experiment (47.1%, 52.0%, 76.9% higher than in the control group ($p<0.001$)). Catalase activity was higher compared with that in the control group only on the 4th and 7th days of the experiment ($p<0.05$ and $p<0.01$, respectively). When a selective mu-OR agonist DAGO was used, by the 7th day, the concentrations of MDA and AHP were significantly lower than those in the control group – by 34.0% and 28.3%, respectively, and the AHP content did not differ significantly from that in the intact rats. By the 7th day, the activity of both antioxidant enzymes was higher than that in control rats: SOD – by 39.9% ($p<0.001$), and catalase – by 31.4% ($p<0.01$).

Simulation of a swimming stress was accompanied by an increased intensity of LPO in plasma and liver tissue of the experimental animals. Plasma concentration of intermediate and final LPO metabolites: AHP and MDA – increased by 125.0% and 39.3%, respectively ($p<0.01$-0.001) 39 hours after the exposure (Table 3). The similar changes were also observed in the liver tissue: an increase in the content of AHP and MDA by 90.7% and 42.3%, respectively ($p<0.001$). On the 4th day of the experiment, there was normalization of concentrations of MDA and AHP in plasma and liver tissue ($p>0.05$). Swimming stress led to an 18.5% increase in SOD activity in plasma 39 hours after the exposure ($p<0.05$). In liver tissue, SOD activity decreased over the same period by 14.7% ($p<0.001$), while catalase activity increased by 11.2% ($p<0.05$). By the 7th day, the indicators returned to normal ($p>0.05$). The injection of all the studied OPs had an antioxidant effect. The most pronounced effect was observed when using the selective mu-OR agonist DAGO: the MDA content after 39 hours did not differ significantly from the similar indicator in the intact rats, and the AHP concentration, although being significantly higher than in the intact group, was 30.9% lower compared with that in the control animals ($p<0.05$). There was a 14.7% decrease in SOD activity ($p<0.05$), and catalase activity was higher than that in the intact group by 11.2% 39 hours after the simulation of swimming stress ($p<0.05$). By the 4th day, the activity of both enzymes did not differ from that of the intact rats. The use of DSLET or dynorphin A (1-13) also caused a decrease in the content of both MDA and AHP in blood plasma, but their effects were less pronounced. The injection of DAGO reduced the concentrations of MDA by 23.0% ($p<0.001$) and of AHP – by 29.4% ($p<0.001$) in the liver tissue compared with those the control group 39 hours after the simulation of swimming stress. On the 4th day of the experiment, the concentration of LPO metabolites did not differ significantly from that in the intact rats ($p>0.05$). SOD activity remained almost unchanged throughout the experiment and was significantly higher than that in the control rats ($p<0.001$) and intact rats ($p<0.001$). After 39 hours, the catalase activity, on the contrary, was lower compared with that in the control (by 30.1%, $p<0.001$) and intact (by 22.3%, $p<0.001$) rats. On the 4th and 7th days, it continued to increase. The use of DSLET and dynorphin A (1-13) also had an antioxidant effect, but the effect was less pronounced compared to that of DAGO.

Simulation of chronic stress of limited mobility was accompanied by a significant increase in the content of LPO metabolites in blood plasma and liver tissue. The concentration of MDA increased in plasma by 90.5%, and in liver tissue – by 106.0% ($p<0.001$). There was also a significant increase in the content of AHP (by 166.1% and 158.3%, respectively, in plasma and liver, $p<0.001$). In control animals, a decrease in SOD activity was observed – by 44.3% in plasma and by 34.4% in the liver tissue ($p<0.001$). Catalase activity also reduced by 24.0% and 26.8% in plasma and liver, respectively ($p<0.001$-0.01). The injection of the studied OPs had an inhibitory effect on the processes of LPO in blood plasma and liver tissue. The most pronounced antioxidant effect in plasma was produced by DAGO – there was a decrease in the content of MDA in blood plasma by 33.9% ($p<0.001$), of AHP – by 47.7% ($p<0.001$), whereas SOD activity increased in plasma by 54.4% ($p<0.001$). The activity of catalase in blood plasma did not change ($p>0.05$).

The administration of dynorphin A (1-13) led to the most pronounced antioxidant effects in liver tissue: the concentration of MDA decreased by 38.6% ($p<0.001$), and of AHP – by 31.5% ($p<0.01$) (Fig. 1). SOD activity increased by 29.9% ($p<0.001$), and catalase activity increased by 24.9% ($p<0.05$). Only the injection of dynorphin A (1-13) resulted in a 15.6% increase of catalase activity in plasma compared with that in the control group ($p<0.05$).

Table 3. Effect of Opioid Peptides on the Content of Malondialdehyde and Acyl Hydroperoxides, Activity of Antioxidant Enzymes in the Liver Tissue of Rats at Different Times after Swimming Stress Modeling (M±m, n=8).

Group \ Indicators	Period after stress simulation	Content of malonic dialdehyde, mcmol/g of tissue protein	Content of Acylhydro-peroxides, su	Catalase activity, mcat/g of tissue protein	Superoxi-dismutase activity, su
Intact		16.8±0.6	5.89±0.30	19.7±0.4	30.7±0.5
Control (stressed)	39 hours	23.9±0.6^{xxx}	11.23±0.32^{xxx}	21.9±0.5^{x}	26.2±0.3^{xxx}
	4 days	17.4±0.8	5.78±0.30	21.5±0.5^{x}	25.9±0.5^{xxx}
	7 days	18.4±0.7	5.39±0.26	19.5±0.6	30.7±1.3
Stress + dynorphin A (1-13) at a dose of 20.1 mcg/kg	39 hours	21.2±0.6***	7.85±0.34***	22.6±0.6	32.0±0.5***
	4 days	16.0±0.3	5.49±0.32	22.5±0.4	27.3±0.5
	7 days	15.30±0.3**	5.45±0.23	17.8±0.3*	34.6±0.4*
Stress+ DAGO at a dose of 6.3 mcg/kg	39 hours	18.1±0.5***	7.93±0.24***	15.3±0.3***	34.9±0.4***
	4 days	15.4±0.4	4.87±0.08*	25.7±0.6***	34.2±0.8***
	7 days	15.6±0.2**	5.15±0.20	34.8±0.5***	34.2±0.4*
Stress + DSLET at a dose of 10.0 mcg/kg	39 hours	19.2±0.4***	7.71±1.56***	25.2±0.5**	35.1±0.4***
	4 days	16.0±0.4	4.93±0.34	22.5±0.4	27.5±1.3
	7 days	15.4±0.2**	5.32±0.17	17.4±0.6*	32.6±0.4

Note: ^{x} – p<0.05, ^{xx} – p<0.01, ^{xxx} – p<0.001 compared with intact animals. * – p<0.05, ** – p<0.01, *** – p<0.001 compared with control animals.

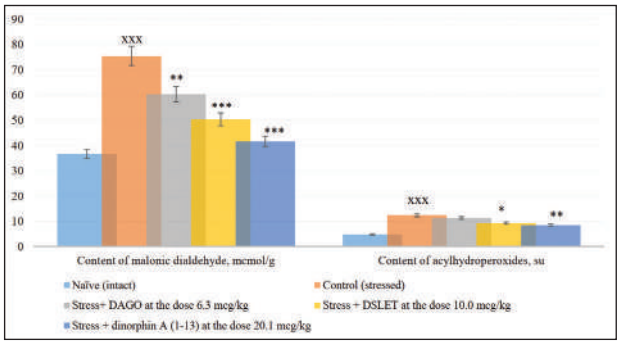

Figure 1. The effect of opioid peptides on the content of lipid peroxidation metabolites in rat liver tissue after modeling chronic stress. Note: ^{xxx} – p<0.001 compared with intact animals. * – p<0.05, ** – p<0.01, *** – p<0.001 compared with control animals.

The study found that all the studied OPs manifest antioxidant activity, which was expressed by a decrease in the content of intermediate and final LPO metabolites: AHP and MDA – in blood plasma and liver tissue under the action of various stress factors. At the same time, some patterns of the peptides effect were established. It was shown that when simulating acute immobilization stress, the use of a selective delta-OR agonist DSLET had the most pronounced effect on the content of MDA and AHP in blood plasma. Thus, it was established that in the animals that had been injected with this peptide on the 4th day after immobilization the concentrations of MDA and AHP did not significantly differ from those in the intact rats. On the 4th day, in the animals that had been exposed to a 12-hour stress, the content of MDA did not significantly differ from the concentration of this substance in the intact group, either.

The most active antioxidant inhibiting the enhancement of LPO processes in the liver tissue was dynorphin A (1-13). The injection of this peptide to rats that had been exposed to 6-hour, 12-hour immobilization or swimming stress, the normalization of the concentration of AHP in the liver tissue was observed as early as on the 4th day after simulating the stress compared with the control group.

Among the studied OPs, DAGO exhibited the greatest antioxidant activity under swimming stress. The concentration of the LPO final product was significantly lower than that in the control group and did not differ from this indicator in the intact rats, both in the liver tissue and in blood plasma 39 hours after stress simulation. The content of AHP was also significantly lower compared with that in the control group. Some patterns of selective OR agonists influencing the activity of antioxidant enzymes: SOD and catalase – under various types of stress were also established. The effects of drugs depended on the nature of the stress exposure and the observation time.

Thirty-nine hours after an acute 6-hour immobilization stress in stress-resistant rats, there was a 2.03-time increase in the number of dystrophically-changed hepatocytes (p<0.001) and a 10.3% decrease in the specific content of normal cells (p<0.001) (Fig. 2). A 3.25-time increase in the specific area of dystrophically-changed sections of the cytoplasm (p<0.001) is also shown. A 34.6% increase in the proportion of binuclear hepatocytes (p<0.001) and a 4.4% decrease in the proportion of nuclei with one nucleolus (p<0.05) indicate the activation of reparative processes in the liver tissue. Disorders of microcirculation in liver tissue were manifested by a 77.4% increase in the total volume of sinusoids (p<0.001). On the 4th day of the experiment, the number of dystrophically-changed cells remained elevated (by 82.4% compared with that in the intact group, p<0.001), and the number of

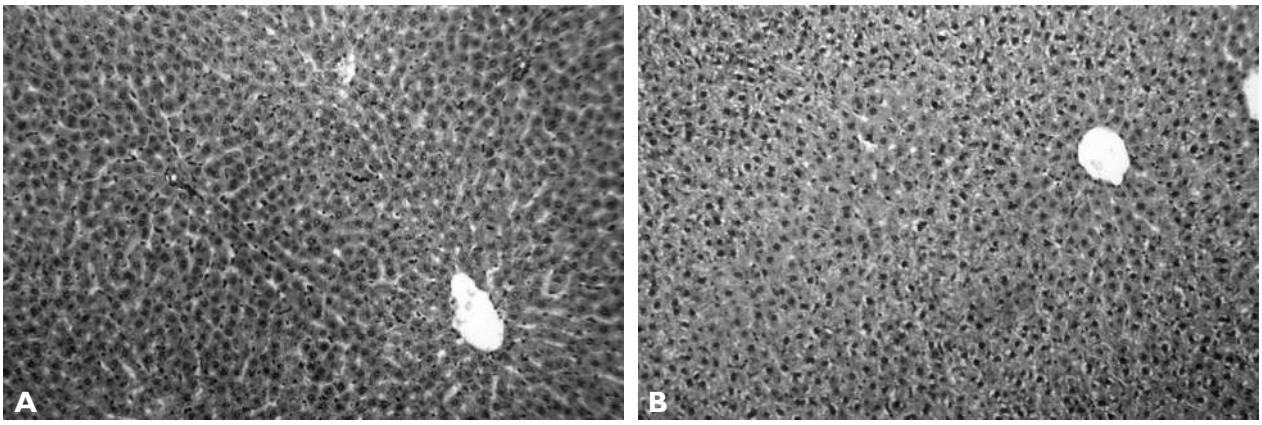

Figure 2. Dystrophic changes of hepatocytes in animals 39 hours after 6-hour immobilization stress. Note: stained with hematoxylin and eosin. **A** – stress-resistant rats, control group: small number of dystrophically-changed hepatocytes, the heterogeneity of the cytoplasm of cells is poorly expressed. × 140. **B** – stress-susceptible rats, control group: numerous dystrophically-changed hepatocytes, the heterogeneity of the cytoplasm of cells is well expressed. × 140.

normal cells decreased (by 8.3%, $p<0.001$). The specific volume of dystrophically-changed sections of the cytoplasm was 2.5 times higher than this indicator in the intact group ($p<0.001$). The share of binuclear hepatocytes was 20.6% higher compared with the previous observation period, which reflects intensification of regenerative processes. The total number of sinusoids was 31.0% higher ($p<0.001$) as compared with that in the intact rats. On the 7th day, all the studied parameters did not differ significantly from those in the intact rats.

Simulation of a 6-hour immobilization stress for stress-susceptible rats led to a 92.8% increase in cells with dystrophic changes in the cytoplasm ($p<0.001$) and an 11.6% decrease in the proportion of normal hepatocytes ($p<0.001$). There was a 39.5% increase in the share of binuclear hepatocytes ($p<0.01$) and a 6.8% decrease in the number of hepatocytes, in the core of which one nucleolus was detected ($p<0.01$). The total number of sinusoids was statistically significantly higher (by 54.9%, $p<0.001$) than that in the intact rats of this group. Four days after stress simulation, the proportion of cells with dystrophic changes in the cytoplasm was significantly higher than that in the intact stress-susceptible animals (by 62.2%, $p<0.001$), and the number of normal hepatocytes was lower than that in the intact group (by 7.8%, $p<0.001$). The specific volume of dystrophically-changed cytoplasm was 3.0 times higher than that in the intact group ($p<0.001$). There was a significant increase in the share of binuclear hepatocytes compared with that in the previous experimental period (by 22.0%) ($p<0.001$). On the contrary, although the number of hepatocytes with one nucleolus increased, however, it was lower than that of the intact stress-susceptible animals (by 4.3%, $p<0.01$). Over that period, the total number of sinusoids also remained higher than that in the intact rats (by 30.8%, $p<0.01$). On the 7th day, like in the group of stress-resistant animals, no indicators differed significantly from those of the intact animals of this group.

The injection of the studied OPs to stress-resistant rats had no significant impact on either the number of normal and dystrophically-changed hepatocytes, or the specific volume of dystrophically-changed sections of the cytoplasm. However, all the studied peptides caused a decrease in the total number of sinusoids compared to that in the control group 39 hours after simulating a six-hour immobilization stress: dynorphin A (1-13) – by 14.1% ($p<0.05$), DAGO – by 12.8% ($p<0.05$), and DSLET – by 15.4% ($p<0.05$). It was found that regenerative processes intensified in the liver parenchyma under the influence of opioids in stress-resistant rats exposed to 6-hour immobilization stress.

Injection of dynorphin A (1-13) caused an increase in the number of binuclear hepatocytes by 25.7% ($p<0.001$), DAGO – by 34.3% ($p<0.001$), DSLET – by 28.0% ($p<0.001$), while a decrease in the proportion of hepatocytes containing one nucleolus was 5.5% when using dynorphin A (1-13), 5.4% – DAGO, and 4.6% – DSLET ($p<0.05$) compared with that in the control group (Fig. 3). After 4 days, a decrease in the total number of sinusoids was observed only in the group of rats that had been treated with dynorphin A (1-13) (by 17.3%, $p<0.05$). The use of DAGO or DSLET did not cause significant changes in this indicator. No intensification of regenerative processes in the groups treated with OP was observed compared with the control group over that period. In the group of stress-resistant rats which had been exposed to a 6-hour immobilization stress and which had been injected with dynorphin A (1-13), the share of binuclear hepatocytes increased by 52.9%, when using DAGO – by 56.5%, and DSLET – by 60.1% ($p<0.001$).

The use of the studied OPs in stress-susceptible rats which had been exposed to a 6-hour immobilization stress also did not significantly affect the number of normal hepatocytes and the number of dystrophically-changed hepatocytes compared with the control group, either. However, the specific volume of dystrophically-changed

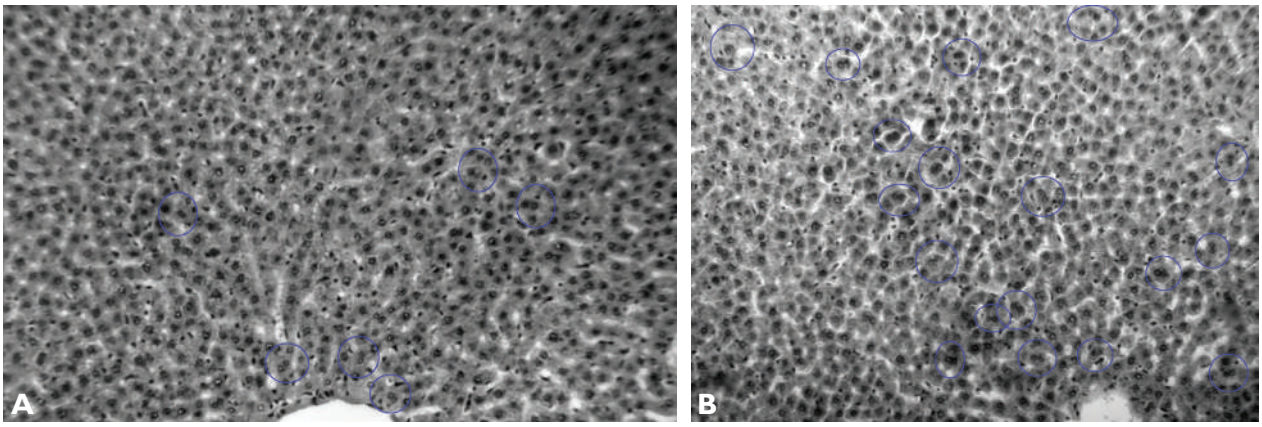

Figure 3. Binuclear hepatocytes in animals 4 days after 6-hour immobilization stress. **Note:** stained with hematoxylin and eosin stain. **A** – stress-susceptible rats, control group: small number of binuclear hepatocytes. × 280. **B** – stress-susceptible rats treated with dynorphin A (1-13): the number of binuclear hepatocytes is significantly higher. × 280.

cytoplasm was statistically significantly lower: when using dynorphin A (1-13) – by 38.1%, DAGO – by 33.3%, and DSLET – by 30.0% ($p<0.001$). There was a decrease in the total number of sinusoids in the groups that had been injected with the opioids under study: when using dynorphin A (1-13) or DSLET – by 13.7% ($p<0.05$). The injection of DAGO did not have a significant effect on this indicator in the stress-susceptible individuals. Intensification of regenerative processes under the influence of opioids in the liver tissue of the stress-susceptible rats which had been exposed to an immobilization stress was confirmed by an increased number of binuclear hepatocytes: with the injection of dynorphin A (1-13) – by 17.9% ($p<0.05$), DAGO – by 23.1% ($p<0.05$), and DSLET – by 24.9% ($p<0.01$) (Fig. 3). However, there was no increase in the proportion of hepatocytes containing a single nucleolus in the opioid groups compared with that in the control animals. On the 4th day of the experiment, there was only a decrease in the total volume of sinusoids with the injection of the peptides: dynorphin A (1-13) – by 16.8% ($p<0.05$), DAGO – by 16.0% ($p<0.05$), and DSLET – by 15.1% ($p<0.05$).Like in the group of stress-resistant animals, on the 7th day of the experiment, there was an increase in the number of binuclear cells in stress-susceptible rats, which had been injected with the studied opioids: when using dynorphin A (1-13) – by 36.2%, DAGO – by 40.9%, and DSLET – by 44.3% ($p<0.001$).

Thus, the study found that simulating six-hour immobilization stress for stress-resistant and stress-susceptible rats was accompanied by the development of dystrophic changes in hepatocytes, impaired microcirculation in the liver tissue, activation of regenerative processes in the parenchyma of the organ as early as 39 hours after the start of the experiment. Only on the 7th day of the experiment, the studied parameters returned to normal. Dystrophic changes in hepatocytes were more pronounced in the stress-susceptible animals, which was manifested by an increase in the specific volume of dystrophically-changed sections of the cytoplasm.

When studying the effect of selective OR agonists on the morphological abnormalities in the liver in rats that had been exposed to a 6-hour immobilization stress, it was found that all the opioids studied had a protective effect, which was manifested by a decrease in the total number of sinusoids and intensification of regenerative processes in the liver parenchyma both in the group of stress-resistant and stress-susceptible animals. In stress-susceptible rats, there was also a decrease in the specific volume of dystrophically-changed cytoplasm when administering peptides. There were no significant differences in the effects of selective agonists of individual classes of OR.

In stress-resistant animals, simulation of long-term mobility restriction stress resulted in a 13.9% decrease in the proportion of normal hepatocytes and a 79.9% increase in the number of dystrophically-changed cells in the cytoplasm ($p<0.001$). The dystrophic changes in the cytoplasm were also more pronounced, which was manifested by a 4.8-time increase in the cytoplasmic vacuolization index ($p<0.001$). Dystrophy of cells was manifested by heterogeneity of the cytoplasm due to its hydration and vacuolization of the cytoplasmic reticulum, and an impaired structure of the plasma membrane. The total number of sinusoids increased by 44.0% ($p<0.01$) (Fig. 4). Kupffer cells lining sinusoids were round, with swollen cytoplasm.

In stress-resistant rats, the development of regenerative processes was observed in the parenchyma of the organ. This was manifested by a 21.7% increase in the average volume of nuclei in rats that had been exposed to stress ($p<0.01$). There was a significant 19.3% decrease in the number of cells, the nucleus of which had only one nucleolus ($p<0.001$). The proportion of binuclear hepatocytes increased by 19.5% ($p<0.05$).

The changes that develop in liver tissue in stress-susceptible animals when simulating chronic stress have a similar direction, but are much more pronounced.

Injection of dynorphin A (1-13) to stress-resistant animals prevented the development of dystrophic chang-

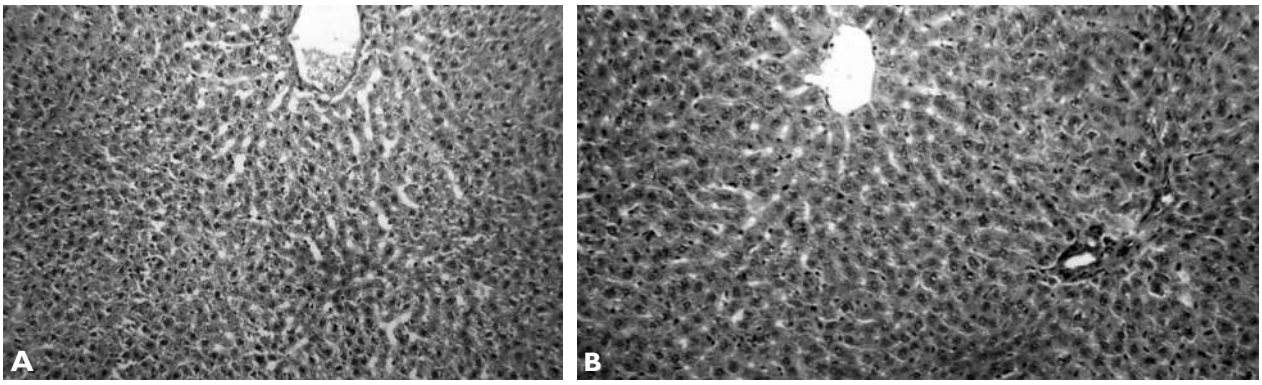

Figure 4. Expansion of the intrahepatic sinuses in animals after chronic limited mobility stress. **Note:** stained with hematoxylin and eosin. **A** – stress-susceptible rats, control group: significant expansion of the intrahepatic sinuses. × 280. **B** – stress-resistant rats, control group: the expansion of the intrahepatic sinuses is less pronounced. × 280.

es in the liver parenchyma, which was manifested by a 22.4% decrease in the number of dystrophically-changed hepatocytes ($p<0.01$). The number of normal hepatocytes also increased by 8.8% ($p<0.01$). The specific area of dystrophically-changed sections of the cytoplasm reduced by 37.5% ($p<0.05$). Dynorphin A (1-13) had no effect on vascular changes in the liver parenchyma. Activation of regenerative processes under the influence of dynorphin A (1-13) in stress-resistant rats was manifested by a 13.3% decrease in the number of cells whose nucleus contained only one nucleolus ($p<0.01$), as well as by a 17.2% increase in the share of binuclear hepatocytes ($p<0.05$). The use of DAGO in stress-resistant rats led to a 11.6% decrease in the proportion of dystrophically-changed hepatocytes ($p<0.05$) and a 5.1% increase in the number of normal cells ($p<0.05$). The use of DSLET did not affect the studied parameters.

The injection of all studied opioids led to a decrease in the severity of destructive disorders and increased regenerative processes in the liver parenchyma of stress-susceptible rats that had been exposed to stress. The most active hepatoprotector was dynorphin A (1-13). The use of this drug led to a 31.6% decrease in the number of dystrophically-changed hepatocytes ($p<0.001$), a 22.4% increase in the proportion of normal cells ($p<0.001$), and a 33.3% decrease in the specific area of the dystrophically-changed sections of the cytoplasm ($p<0.01$). Activation of regenerative processes was manifested by a 30.4% increase in the average size of hepatocyte nuclei ($p<0.01$), as well as by a 20.0% decrease in the proportion of cells with only one nucleolus in the nucleus ($p<0.001$).

The use of DAGO or DSLET in stress-susceptible rats that had been exposed to a prolonged stress had a protective effect on the liver cells and stimulated the regenerative processes in the parenchyma. When injecting DAGO, there was a 13.5% increase in the number of normal hepatocytes ($p<0.01$), a 19.0% decrease in the proportion of dystrophically-changed cells ($p<0.01$), and a 41.2% decrease in the specific proportion of dystrophically-changed sections of the cytoplasm ($p<0.01$). A 13.6% increase in the proportion of binuclear hepatocytes ($p<0.05$) and an 11.8% decrease in the proportion of cells with only one nucleolus in the nucleus ($p<0.01$) indicated the activation of regenerative processes in the parenchyma of the liver under the influence of the peptide. Unlike stress-resistant rats, the injection of DSLET to stress-susceptible rats also led to a 15.9% decrease in the number of dystrophically-changed hepatocytes ($p<0.05$), an 11.3% increase in the proportion of normal cells ($p<0.05$), and a 33.3% decrease in the specific the area of dystrophically-changed sections of the cytoplasm ($p<0.01$). The effect of this peptide on regeneration was significantly lower than that of other opioids. There was recorded only a 7.3% decrease in the proportion of cells whose nucleus contained one nucleolus ($p<0.05$).

It was established that in the rats that had been exposed to chronic stress, like when simulating an acute immobilization stress, there were both manifestations of liver tissue damage and manifestations of tissue regeneration. The changes in liver tissue damage were more pronounced in the stress-susceptible animals.

The studied OPs had a stress-limiting hepatoprotective effect, leading to a decrease in dystrophic changes in the liver tissue and activation of reparative processes. The most pronounced hepatoprotective effect in both acute and chronic stress was shown by selective kappa-OR agonist dynorphin A (1-13).

The investigation did not establish statistically significant differences in the effects of various types of stress on the biochemical parameters in stress-resistant and stress-susceptible rats, so the data are presented for the entire group of animals.

Simulating an acute 3-hour stress led to an increase in AST activity by 32.6% ($p<0.001$) and in ALT – by 49.4% ($p<0.001$) 39 hours after the end of immobilization. As early as on the 4th day of the experiment, AST and ALT activity returned back to normal in blood plasma of the experimental animals. In the rats that had been exposed to an acute 6-hour immobilization stress, there was a 2.51-time increase in AST activity ($p<0.001$) and a 2.67-

time increase – in ALT ($p<0.001$) only 39 hours after the experiment (Table 4). There was a 5.0% decrease in the total protein content ($p<0.05$). Acute 12-hour immobilization stress was accompanied by a 2.69-time increase in AST activity and a 2.85-time increase in ALT activity ($p<0.001$) 39 hours after the end of exposure. It was also established that the concentration of total protein in blood plasma decreased by 5.3% ($p<0.05$). On the 4th day after the stress exposure, the AST and ALT activities remained elevated (by 27.0% and 34.5%, respectively, $p<0.01$) compared to those in the intact rats. There was a 7.8% decrease in the albumin content ($p<0.05$). On day 7th, all the studied parameters in the intact and control groups did not differ.

The use of all the studied OPs reduced the severity of cytolytic syndrome, which was manifested by lower values of AST and ALT activity, in case of immobilization of various duration. In the rats which had been exposed to a 3-hour or 6-hour immobilization stress the most pronounced anti-stress effect was observed with the use of selective agonists of kappa and delta-OR dynorphin A (1-13) and DSLET.

The use of a selective mu-OR agonist DAGO at a dose of 6.3 μg/kg in a 6-hour stress also caused a decrease in the activities of AST and ALT compared with those in the control group, but to a lesser extent than with dynorphin A (1-13) or DSLET: AST activity decreased by 18.5% ($p<0.01$) and ALT – by 28.7% ($p<0.01$). The use of DAGO also prevented a decrease in the total protein content in blood plasma 39 hours after stress simulation.

Simulation of swimming stress was accompanied by an increase in AST activity in blood plasma by 13.9% ($p<0.01$) only 39 hours after the stress modeling. The use of OP DSLET and dynorphin A (1-13) had a hepatoprotective effect, which was manifested by a decrease in AST activity 39 hours after the simulation of swimming stress by 17.7% and 19.2%, respectively ($p<0.001$). At the same time, AST activity was not different from that of the intact rats. On the 4th and 7th days of the experiment, there were no differences in all the studied parameters between the control rats and the animals that had been injected with OP.

Simulation of the twelve-day limited mobility stress was accompanied by the development of cytolytic syndrome and impaired protein-synthetic liver function (Table 5). Thus, AST activity in blood plasma increased 2.5 times ($p<0.001$) and ALT – 3.3 times ($p<0.001$). There was a decrease in the content of total protein by 5.0% ($p<0.01$) and albumin – by 9.0% ($p<0.001$).

The use of OPs prevented the suppression of protein-synthetic liver function, which was manifested by the absence of differences between the values of the indicators of total protein and albumin in the group of the intact rats and stressed animals that had received OPs. All the studied peptides reduced the severity of cytolytic syndrome: for instance, with the injection of dynorphin A (1-13), AST activity decreased by 33.5% ($p<0.001$) and ALT – by 48.6% ($p<0.001$). DSLET and DAGO had a similar effect: the use of DSLET was accompanied by a decrease in AST activity by 25.4% ($p<0.001$) and ALT – by 45.7% ($p<0.001$), and the use DAGO resulted in a decrease in AST by 17.4% ($p<0.01$) and ALT – by 17.5% ($p<0.05$).

Thus, it was found that the use of OPs reduced the severity of cytolytic syndrome and prevented the disorders of the protein-synthetic function of the liver in the animals which had been exposed to an acute or chronic stress.

Simulation of acute immobilization stress was accompanied by an increase in the concentrations of TC, TG, LDL, VLDL, NEFA 39 hours after the end of immobilization compared with those in the intact animals (Table 6). On the 7th day after the immobilization simulation, no statistically significant differences in the studied parameters were found between the intact group and the animals that had been exposed to 3- or 6-hour stress. In the rats that had been exposed to a 12-hour stress, the concentration of TC was significantly lower compared with that in the intact animals (by 20.9%, $p<0.01$).

The use of OPs in the rats that had been exposed to 3-hour immobilization led to a decrease in the concentrations of TG, LDL, NEFA only 39 hours after the end of stress compared with those in the control group. For instance, the decrease in TG concentration when administering DAGO was by 27.0% ($p<0.001$), DSLET – by 30.4% ($p<0.001$), and dynorphin A (1-13) – by 29.6% ($p<0.001$). The decrease in the content of LDL in all groups that had been injected with peptides was the same – by 28.6% ($p<0.05$). The decrease in the concentration of NEFA was the following: when using DAGO or DSLET – by 28.4% ($p<0.01$), and when using dynorphin A (1-13) – by 33.1% ($p<0.01$). The increase in HDL concentration in rats treated with DAGO was 39.3% ($p<0.01$), and with dynorphin A (1-13) – 42.9% ($p<0.01$).

In the animals that had been exposed to a 6-hour simulated stress, 39 hours after the application of DAGO or DSLET, there was found a decrease in the contents of the following: TC – by 21.2% and 18.6%, respectively ($p<0.01$), TG – by 23.2% and 21.0% respectively ($p<0.05$), and NEFA – by 15.0% and 20.3%, respectively ($p<0.05$). In both groups, there was also an increase in HDL concentration: with the injection of DAGO – 3.2 times, and of DSLET – 4.9 times ($p<0.001$). In the group treated with dynorphin A (1-13), a decrease in the content of NEFA was observed – by 20.9% ($p<0.05$), as well as a 4.2-time increase in HDL ($p<0.001$). On the 4th day, there was a decrease in TG by 16.1%, 15.6% and 17.4%, as well as a decrease in NEFA by 26.8%, 34.1% and 30.1% in all the experimental groups, which had been injected with the investigated OPs: DAGO, DSLET or dynorphin A (1-13), respectively (p <0.05-0.01). There was an increase in the concentration of HDL only in the groups that had been injected with DAGO (by 35.5%, $p<0.05$) or DSLET (by 25.0%, $p<0.05$). On the 7th day after simulating the 6-hour immobilization stress, there was a decrease in the levels of VLDL and LDL and an increase in the concentration of in all the groups receiving OPs.

Table 4. The Effect of 6-hour Immobilization Stress on the Content of ALT and AST in Blood Plasma of Rats (M±m, n=8).

Indicator / Group	Period after stress simulation	Aspartate aminotransferase, un/L	Alanine aminotransferase, un/L
Intact		193.0±11.6	53.4±3.3
Control (stressed)	39 hours	484.8±18.8^{xxx}	142.5±10.8^{xxx}
	4 days	213.4±10.3	54.8±2.5
	7 days	206.6±19.7	52.8±5.2
Stress+ DAGO at a dose of 6.3 mcg/kg	39 hours	395.1±8.8**	101.6±3.5**
	4 days	195.4±5.2	57.4±2.1
	7 days	199.8±12.2	47.8±1.6
Stress + DSLET at a dose of 10.0 mcg/kg	39 hours	303.5±13.1***	92.1±3.2***
	4 days	201.5±6.3	55.9±2.2
	7 days	198.4±8.8	51.4±1.3
Stress + dynorphin A (1-13) at a dose of 20.1 mcg/kg	39 hours	298.5±11.1***	80.8±2.4***
	4 days	198.4±6.4	55.6±2.5
	7 days	203.0±12.5	54.6±2.1

Note: ^{x} – p<0.05, ^{xx} – p<0.01, ^{xxx} – p<0.001 compared with intact animals. * – p<0.05, ** – p<0.01, *** – p<0.001 compared with control animals.

Table 5. The Effect of Chronic Stress on the Activity of ALT and AST in Blood Plasma of Rats (M±m, n=8).

Indicator / Group	Aspartate aminotransferase, un/L	Alanine aminotransferase, un/L
Intact	205.8±5.1	56.0±2.4
Control (stressed)	518.9±12.4^{xxx}	184.1±9.2^{xxx}
Chronic stress+ DAGO at a dose of 6.3 mcg/kg	428.8±11.7**	151.9±7.4*
Chronic stress + DSLET at a dose of 10.0 mcg/kg	386.9±13.0***	99.9±5.9***
Chronic stress + dynorphin A (1-13) at a dose of 20.1 mcg/kg	344.9±9.0***	94.5±4.0***

Note: ^{x} – p<0.05, ^{xx} – p<0.01, ^{xxx} – p<0.001 compared with intact animals. * – p<0.05, ** – p<0.01, *** – p<0.001 compared with control animals.

Table 6. The Effect of Opioid Peptides on the Content of Lipoproteins of Different Density in Blood Plasma of Rats at Different Periods After 6-hour Immobilization (M±m, n=8).

Indicator / Group	Period after stress simulation	LPVLD, mmol/L	LPLD, mmol/L	LPHD, mmol/L
Intact		0.07±0.01	0.06±0.01	0.14±0.01
Control (stressed)	39 hours	0.14±0.01 ^{xxx}	0.14±0.01^{xxx}	0.14±0.02
	4 days	0.08±0.01	0.06±0.01	0.40±0.03 ^{xxx}
	7 days	0.09±0.01	0.08±0.01	0.15±0.02
Stress+ DAGO at a dose of 6.3 mcg/kg	39 hours	0.12±0.01	0.13±0.01	0.45±0.05***
	4 days	0.10±0.01	0.08±0.01	0.54±0.03*
	7 days	0.06±0.01*	0.05±0.01*	0.37±0.02***
Stress + DSLET at a dose of 10.0 mcg/kg	39 hours	0.13±0.01	0.14±0.01	0.69±0.04***
	4 days	0.05±0.01	0.04±0.01	0.50±0.02*
	7 days	0.06±0.01*	0.05±0.01*	0.43±0.04***
Stress + dynorphin A (1-13) at a dose of 20.1 mcg/kg	39 hours	0.15±0.01	0.14±0.01	0.59±0.04***
	4 days	0.06±0.01	0.04±0.01	0.44±0.02
	7 days	0.05±0.01*	0.04±0.01*	0.50±0.03***

Note: ^{x} – p<0.05, ^{xx} – p<0.01, ^{xxx} – p<0.001 compared with intact animals. * – p<0.05, ** – p<0.01, *** – p<0.001 compared with control animals.

Thirty-nine hours after 12-hour immobilization in the groups that had been treated with the studied OPs, there was a decrease in the TC and TG contents in comparison with those in the control group. For instance, the concentration of TC decreased by 22.6% (p<0.001), and of TG – by 18.8% (p<0.01) in the group that had been treated with DAGO. The use of DSLET and dynorphin A (1-13) was accompanied by a drop in the content of not only TC and

TG, but also of NEFA: when using DSLET – by 20.2% ($p<0.001$), 13.1% ($p<0.05$) and 19.1% ($p<0.05$), respectively, and when using dynorphin A (1-13) – by 22.6% ($p<0.001$), 18.8% ($p<0.001$) and 22.1% ($p<0.05$), respectively, compared with those in the control group.

There were no changes in the concentration of VLDL and LDL, while the content of HDL significantly increased – 2.2 times ($p<0.01$), 2.4 times ($p<0.001$) and 3.1 times ($p<0.001$), respectively, when using DAGO, DSLET or dynorphin A (1-13). On the 4th day after the simulation of the 12-hour immobilization stress, the concentrations of TG and NEFA were significantly lower compared with those in the control group in all the groups, which had been given OPs. In the rats treated with DAGO, the decrease was by 21.1% and 26.2% ($p<0.05$, $p<0.01$), with DSLET – by 29.8% and 33.8% ($p<0.01$), and with dynorphin A (1-13) – by 25.2% and 34.6% ($p<0.01$–$p<0.001$), respectively. The HDL content was also still significantly higher than that in the control group: when DAGO was administered – by 45.5% or when dynorphin A (1-13) was administered – by 45.5% ($p<0.05$). On the 7th day, the concentration of HDL in the groups treated with OPs was significantly higher than that in the control group ($p<0.01$-0.001).

Simulation of 2-hour swimming stress in Wistar rats caused increased levels of the following in plasma: of TC – by 25.7% ($p<0.001$), of NEFA – by 47.2% ($p<0.01$), of TG – by 19.6% ($p<0.01$), of LDL – by 77.8% ($p<0.001$), and of HDL – by 36.8% ($p<0.01$) –39 hours after the exposure (Table 7). On the 4th day of the experiment, the indicators returned back to normal. At the same time, though the content of HDL was reducing, it still remained significantly higher than that in the intact animals (by 26.3%, $p<0.01$). On the 7th day, the concentration of HDL remained 21.1% higher than that of the intact rats ($p<0.05$).

The injection of any of the studied OR agonists reduced the stress-induced changes in lipid metabolism in the animals which had been exposed to swimming stress. Dynorphin A (1-13) and DAGO had the most pronounced effect. There were no differences in the contents of TC, NEFA, TG, and LDL cholesterol in blood plasma between the stressed rats receiving one of these preparations and intact animals ($p>0.05$) when using these peptides as early as 39 hours after the simulation of swimming stress.

Only DSLET resulted in an increase in HDL levels in blood plasma in the stressed rats compared those in with the control group. The concentration of HDL was 65.4% higher ($p <0.01$) compared with that in the control group 39 hours after the exposure. The injection of neither DAGO nor dynorphin A (1-13) caused significant changes in HDL concentrations at all experimental periods compared with those in the control group.

Simulation of 12-day stress of limited mobility caused a statistically significant increase in the contents of TC, TG and NEFA ($p<0.01$–0.001) in plasma compared with those in the intact rats (by 70.2%, by 42.6% and by 174.6%, respectively). The change in the contents of lipoproteins was multidirectional: whereas the concentrations of VLDL and LDL increased by 166.7% and 150%, respectively, the content of HDL, by contrast, decreased by 40.7% ($p<0.01$-0.001) (Table 8).

The use of Ops – DSLET or DAGO – caused a significant decrease in the concentrations of TC, TG and NEFA. At the same time, the effect of DSLET was more pronounced, which appeared in a decrease in the concentration of not only TC and NEFA, but also of TG ($p<0.05$).

The studied OPs did not significantly affect the contents of LDL and VLDL in blood plasma of the rats that had been exposed to chronic stress ($p>0.05$). However, the concentration of HDL in plasma of the rats treated with peptides was significantly higher than that in plasma of the control ($p<0.01$). However, no differences were found in the effects of individual opioids.

Thus, it was established that stress simulation was accompanied by an increase in the contents of TC, TG, NEFA, VLDL and LDL in blood plasma of rats, the severity and duration of the increase depended on the severity of the stress. Changes in HDL production depended on the severity of stress. Stress-limiting effect of OPs was accompanied by a decrease in the concentration of TC, TG, NEFA, VLDL and LDL, as well as an increase in the content of HDL. A more pronounced effect on stress-induced changes in lipid metabolism was provided by delta-OR agonist DSLET and kappa-OR agonist dynorphin A (1-13).

The data obtained show that in rats of the control group on the 1st day after resection of 70% of the liver, there was an increase in the mass of the right and caudate lobes by 20.8%, and the relative weight of the regenerating liver was 36.3% of the initial mass of the organ (before resection). On the 3rd, the mass of the two lobes remaining after resection increased by 128.7%, and the relative mass of the liver increased to 68.7%. On 7th day, the relative weight of the organ was 86.2% of the original weight due to the active regeneration of the right and caudate lobes (by 187.3%).

The use of OPs had a stimulating effect on the regeneration of liver tissue after PHE. One day after resection, the mass of the right and caudate lobes increased by 53.3% in the group treated by DAGO, by 48.0% – in the group treated by dynorphin A (1-13), and by 38.3% – in the group treated by DSLET. These values were significantly higher than the same indicators in the control group: when injecting DAGO – by 156.3% ($p<0.001$), dynorphin A (1-13) – by 130.8% ($p<0.001$), and DSLET – by 84.1% ($p<0.05$). In the groups of animals treated with OPs, there was also a significant increase in the relative weight of the organ: when injecting DAGO – by 46.0%, dynorphin A (1-13) – by 44.4%, and DSLET – by 41.5%. These indicators were also significantly higher than those in the rats of the control group: when injecting DAGO – by 26.7% ($p<0.001$), dynorphin A (1-13) – by 22.3% ($p<0.001$), and DSLET – by 14.3% ($p<0.05$).

Three days after PHE in the groups of animals treated with dynorphin A (1-13) or DSLET, there was neither

Table 7. The Effect of Opioid Peptides on the Content of Lipoproteins of Different Density in Blood Plasma of Rats at Different Periods of Swimming Stress Simulation (M±m, n=8).

Indicator / Group	Period after stress simulation	VLDL, mmol/L	LDL, mmol/L	HDL, mmol/L
Intact		0.08±0.01	0.09±0.01	0.19±0.01
Control (stressed)	39 hours	0.14±0.01 ^{xxx}	0.16±0.01^{xxx}	0.26±0.01^{xx}
	4 days	0.12±0.01	0.11±0.01	0.24±0.02^{xx}
	7 days	0.09±0.01	0.10±0.01	0.23±0.02^{x}
Stress+ DAGO at a dose of 6.3 mcg/kg	39 hours	0.12±0.01	0.12±0.01	0.31±0.02
	4 days	0.13±0.01	0.11±0.01	0.25±0.01
	7 days	0.12±0.01	0.11±0.01	0.27±0.02
Stress + DSLET at a dose of 10.0 mcg/kg	39 hours	0.11±0.01	0.13±0.02	0.43±0.03**
	4 days	0.09±0.01	0.10±0.01	0.23±0.01
	7 days	0.09±0.01	0.10±0.01	0.21±0.02
Stress + dynorphin A (1-13) at a dose of 20.1 mcg/kg	39 hours	0.12±0.01***	0.11±0.01***	0.31±0.02
	4 days	0.10±0.01	0.11±0.01	0.30±0.02
	7 days	0.11±0.01	0.10±0.01	0.25±0.02

Note: ^{x} – p<0.05, ^{xx} – p<0.01, ^{xxx} – p<0.001 compared with intact animals. * – p<0.05, ** – p<0.01, *** – p<0.001 compared with control animals.

Table 8. The Effect of Opioid Peptides on the Content of Lipoproteins of Different Density in Blood Plasma of Rats at Different Periods of Chronic Stress Simulation (M±m, n=8).

Indicator / Group	VLD, mmol/L	LDL, mmol/L	HDL, mmol/L
Intact	0.06±0.01	0.06±0.01	0.27±0.02
Control (stressed)	0.16±0.01^{xxx}	0.15±0.01^{xxx}	0.16±0.01^{xx}
Stress+ DAGO at a dose of 6.3 mcg/kg	0.13±0.01	0.14±0.01	0.34±0.04**
Stress + DSLET at a dose of 10.0 mcg/kg	0.14±0.01	0.14±0.01	0.35±0.05**
Stress + dynorphin A (1-13) at a dose of 20.1 mcg/kg	0.14±0.01	0.14±0.01	0.33±0.04**

Note: ^{x} – p<0.05, ^{xx} – p<0.01, ^{xxx} – p<0.001 compared with intact animals. * – p<0.05, ** – p<0.01, *** – p<0.001 compared with control animals.

statistically significant increase in the relative mass of the right and caudate lobes, nor in the relative mass of the liver. Only with the injection of DAGO, the relative mass index of the right and caudate lobes increased by 25.6% (p<0.01). Accordingly, only in this group an increase in the relative mass of the liver was noted – by 14.2% (p<0.01).

On the 7th day of the experiment, in all the groups of rats that had undergone PHE and received OPs, there was a statistically significant increase in the relative weight of the right and caudate lobes, as well as in the relative weight of the liver. When using DAGO, the relative mass of the right and caudate lobes increased by 235.8%, which was 25.9% higher compared with that in the control group (p <0.001), when using dynorphin A (1-13) – by 278.2% (48.5% higher than that in the control group, p<0.001), when using DSLET – by 222.7% (18.9% higher than that in the control group, p<0.01). On the 7th day a complete recovery of liver mass in the animals treated with DAGO was recorded. This indicator was 100.7%, which was 16.8% higher than that in the control animals (p<0.001). In the group of rats treated with dynorphin A (1-13), there was a slight excess of the initial liver mass – 113.5%, which was 31.7% higher than that in the control animals (p<0.001). Only in the animals that had been treated with DSLET, the relative weight of the liver did not reach the initial level – 96.8%, but this was also significantly higher than that in the control group (by 12.3%, p<0.01).

Preliminary simulation of 12-day chronic stress for Wistar rats did not affect the regeneration of the rats' liver after PHE. One day after resection, a stimulating effect of the immobilization stress was recorded on regenerative processes in the liver after PHE: a more significant increase in the mass of the right and caudate lobes was found one day after surgery (by 187.5%, p<0.001) compared with that in the non-stressed animals. The relative weight of the liver also increased significantly – by 32.0% (p<0.001). However, on the 3rd and 7th days, there were no statistically significant differences in both studied parameters (p>0.05) between the stressed and non-stressed animals that had undergone PHE according. The injection of DAGO to Wistar rats which had been exposed to stress and the subsequent 70% hepatectomy did not significantly affect the regeneration processes in the liver at all the

periods of observation. The use of dynorphin A (1-13) had a stimulating effect on liver regeneration only on the 7th day of the experiment. During that period, recovery of the liver mass was observed (100.3% of the initial mass of the organ) and the relative weight of the liver was statistically significantly higher than that in the rats of the control group (by 11.7%, $p<0.001$). The injection of DSLET caused an increase in the studied indicators in the stressed rats only on the 1st day of the experiment. For instance, the relative increase in the mass of the right and caudate lobes turned out to be 47.5% higher than that in the control group ($p<0.05$), and the relative mass of the liver was 22.3% higher ($p<0.01$).

Simulation of 70% hepatectomy caused an increase in the activities of ALT, AST and LDH in blood plasma on the 1st day after surgery by 120.5%, 59.8% and 76.0%, respectively ($p<0.001$) (Table 9). On the 3rd day after surgery, the activities of ALT and AST increased compared to those on the first day of observation and remained significantly higher than such indicators in the intact rats ($p<0.001$). The activity of LDH was 65.2% higher than that in the intact animals ($p<0.001$). During this period, the disorders of the protein-synthetic function of the liver were observed, which was manifested by a decrease in the concentrations of total protein (by 7.0%, $p<0.01$) and albumin (by 11.3%, $p<0.001$). There was a 26.6% increase in total bilirubin in blood plasma ($p<0.001$). ALT, AST and LDH activities in the control animals were still higher than those in the intact rats (by 80.1%, 29.2% and 26.4%, respectively, $p<0.05$-0.001) on the 7th day after resection. The concentrations of total protein and albumin were lower than those in the animals of the intact group (by 5.3% and 15.5%, respectively, $p<0.05$-0.001). There were no differences between the levels of total bilirubin in plasma of the animals of the control and intact groups 7 days after surgery.

The injection of the studied OPs did not affect the indicators of cytolytic syndrome, protein-synthetic and detoxification functions of the liver in animals which had undergone 70% liver resection on the first day after surgery. On the 3rd day, the peptides exhibited their hepatoprotective effect. So, in the rats that were injected with dynorphin A (1-13), the activities of ALT, AST and LDG were lower by 32.5%, 7.3% and 18.3%, respectively ($p<0.05$-0.001) compared with those in the control group, when using DAGO – by 29.9%, 9.0% and 21.8% lower ($p<0.05$-0.001), and when using DSLET - by 28.4%, 11.0% and 21.4% lower, respectively ($p<0.05$-0.001). An increase in the concentration of total protein by 2.9%, 3.2% and 3.0% in the rats treated by dynorphin A (1-13), DAGO and DSLET, respectively, was also found ($p<0.05$). There was a decrease in the level of total bilirubin in blood plasma on the 3rd day after liver resection when using dynorphin A (1-13), DAGO and DSLET – by 6.0%, 11.0% and 6.0%, respectively ($p<0.05$). In the group of animals which had undergone liver resection, administration of dynorphin A (1-13) caused a decrease in the activities of ALT, AST, LDG by 32.8%, 27.0% and 18.3%, respectively, compared to those in the control group 7 days after surgery ($p<0.001$). An increase in the concentrations of total protein (by 5.8%, $p<0.01$) and albumin (by 8.4%, $p<0.01$) indicates an increase in the protein-synthetic function of the liver under the influence of the peptide. The injection of DAGO decreased the activities of ALT, AST and LDH by 33.7%, 25.6% and 20.3%, respectively, compared to those in the control group ($p<0.001$). There was an increase in the contents of total protein and albumin by 6.9% ($p<0.001$) and 13.0% ($p<0.001$), respectively. A similar effect resulted from the use of DSLET. The injection of this peptide decreased ALT activity by 35.2% ($p<0.001$), AST – by 29.0% ($p<0.001$), and LDG – by 22.6% ($p<0.001$). An increase in the total protein content (by 6.6%, $p<0.001$) and albumin content (by 12.6%, $p<0.001$) was also observed. The study showed that the preliminary simulation of long-term stress did not affect the changes in the activities of ALT, AST and LDG, the concentrations of total protein, albumin and total bilirubin in the animals that had undergone PHE throughout the experiment compared with the non-stressed rats. Of all the OPs used in the study, only DAGO showed a cytoprotective effect in stressed rats which had undergone PHE, which was manifested by a decrease in the activity of the following in blood plasma on the 4th day after the operation: of ALT – by 20.6% ($p<0.05$), of AST – by 8.0 % ($p<0.05$), and of LDG – by 28.6% ($p<0.01$). On the 7th day after PHE, the activities of ALT and LDG in plasma of rats that had undergone resection and had been treated with DAGO was lower by 28.8% and 17.4%, respectively, compared with those in the non-stressed rats after PHE treated with DAGO.

The obtained results confirm the literature data on the nearly complete recovery of the liver mass after 70% hepatectomy in rats after 7–10 days (in the control group, the relative weight of the liver was 86.2%). The use of OPs had a stimulating effect on the regeneration of the liver after PHE. The most pronounced effect was shown by the selective kappa-OR agonist dynorphin A (1-13) and the selective mu-OR agonist DAGO. As early as one day after PHE, the relative weight of the liver in the rats treated with OPs was significantly higher than that in the animals of the control group. A more pronounced stimulating effect of DAGO appeared on the 3rd day after surgery. It was in the rats treated with this peptide, where there was an increase in the relative weight of the liver and the relative weight of the right and caudate lobes. The stimulating effect of dynorphin A (1-13) was most pronounced on the 7th day of the experiment, when the relative weight of the liver in the rats receiving it was 113.5%. In the group of animals treated with DAGO, the restoration of the initial mass of the liver was also observed, and in the rats that were injected with DSLET the restoration was nearly complete.

It is known that the process of liver regeneration after PHE is controlled by a whole cascade of growth factors, cytokines, transcription factors; however, the most important of them are hepatocyte growth factor (HGF),

Table 9. The Effect of PHE on the Activity of ALT, AST and LDH in Blood Plasma of Rats (M±m, n=8).

Indicator / Group	Period after resection	Alanine aminotransferase, un/L	Aspartate aminotransferase, un/L	Lactate-dehydrogenaze, un/L
Intact		52.3±3.7	195.7±13.3	244.3±4.8
Control (stressed)	1 days	115.3±5.5^{xxx}	312.7±8.1^{xxx}	430.0±17.2^{xxx}
	3 days	133.3±5.6^{xxx}	342.7±8.8^{xxx}	403.7±10.4^{xxx}
	7 days	94.2±5.5^{xxx}	252.8±10.8^{x}	308.7±7.2^{x}
Stress+ DAGO at a dose of 6.3 mcg/kg	1 days	98.7±5.6	300.2±8.5	432.0±8.6
	3 days	93.5±5.0***	311.8±7.0*	315.7±7.2***
	7 days	62.5±2.9***	188.0±6.9***	246.0±6.7***
Stress + DSLET at a dose of 10.0 mcg/kg	1 days	100.2±7.3	289.5±11.5	418.3±10.0
	3 days	95.5±3.6***	309.0±10.1*	317.2±13.6***
	7 days	61.0±3.3***	179.5±4.4***	238.8±5.4***
Stress + dynorphin A (1-13) at a dose of 20.1 mcg/kg	1 days	103.2±5.6	284.0±10.7	414.7±10.00
	3 days	90.0±3.9***	317.5±6.5*	329.7±10.9**
	7 days	63.3±3.0***	184.5±6.0***	252.2±7.8***

Note: ^{x} – p<0.05, ^{xx} – p<0.01, ^{xxx} – p<0.001 compared with intact animals. * – p<0.05, ** – p<0.01, *** – p<0.001 compared with control animals.

epidermal growth factor, transforming growth factor alpha, and angiogenic factors (Alvarado et al. 2016, Beier et al. 2016, E et al. 2017, Ibis et al. 2017, Kirschbaum et al. 2017, Lisman and Porte 2016). Hepatocyte growth factor plays a key role because it induces the transition of hepatocytes to the mitotic phase. In an inactive state, it is present in the liver tissue matrix in significant quantities, and after hepatectomy, under the influence of urokinase and the enzymes of the metalloprotease family, HGF gets activated and triggers the division of hepatocytes. Of interest if the fact that the epidermal growth factor acts mainly at the initial stage of liver regeneration, while the transforming growth factor alpha – at later stages (Fausto et al. 2012, Adamek et al. 2017).

It is assumed in this study that the stimulating effect of OPs on liver regeneration after PHE is associated with their effect on the cytokine cascade, which leads to either an increase in the concentration of active HGF in liver tissue or to acceleration of its activation, which leads to the early onset of mitotic division of the remaining cells. DAGO has the greatest effect, which is why its stimulating effect is observed at all periods of observation. Dynorphin A (1-13) can activate the release of a transforming growth factor alpha more than other OPs, therefore its activating effect on the regeneration of the liver is most pronounced at the final stage (on the 7th day).

Simulation of 12-day chronic limited mobility stress in rats caused activation of the liver tissue regeneration process on the first day after 70% hepatectomy. Further - on the 3rd and 7th days after resection, this effect is not observed. There were no changes in the functional activity of the liver.

It is assumed in this study that this is due to the fact that along with stress-induced lesions of hepatocytes, there is activation of regenerative processes in the liver tissue after stress. That is, there is activation of hepatocyte growth factor and mitotic cell division. Therefore, surviving hepatocytes begin to divide faster after 70% of hepatectomy, against the background of a high level of active HGF.

When conducting a factor analysis, simulation of 3-hour acute stress led after 39 hours to the formation of 3 factors. The first factor, having the largest share of the total variance of the factor and a large share of the total variance, included the following indicators: contents of MDA and total protein in plasma had a positive factor load, AST activity and AHP concentration in plasma, as well as catalase and SOD activities in the liver had a negative factor load. The second factor included indicators characterizing the contents of HDL, TG and VLDL. The third factor included correlated indicators of SOD and catalase activities in plasma.

As the stress load increased, the factor load included lipid and protein metabolism indicators. So with a 6-hour immobilization stress, 39 hours after stress simulation, the first factor included indicators of catalase activity and the concentration of AHP in plasma with a positive load and the contents of VLDL and HDL with a negative one. The second factor included the contents of total protein, albumin in plasma and contents of TG, LDL, and NEFA. During other periods of observation, the factor load did not change significantly. After simulating the 12-hour immobilization stress, the first factor included the positive load indicators – LDL, VLDL, plasma albumin content and the negative load — MDA and AGP concentrations in plasma. By the 7th day, the factor structure changed, and the first factor included the indicators of the levels of TG, HDL, catalase activity in plasma and liver tissue, ALT activity, MDA concentration in liver tissue and blood plasma. These changes can indicate the activation of lipolysis and LPO, as the leading processes under stress. The injection of all OPs changed the factor load. At the same time, the structure of factors was more pronounced with the injection of DSLET or dynorphin A (1-13).

When simulating chronic stress, the first factor included the indicators of catalase activity in plasma, the content of AHP in plasma and liver tissue, the activity of SOD in plasma, the contents of VLDL and NEFA. A similar factor structure was with the administration of dynorphin A (1-13) and DSLET. With the administration of DAGO, the first factor included the indicators of TG content, the contents of MDA and HDL in blood plasma.

The structure of the factors during swimming stress includes only the indicators of LPO in plasma and liver tissue, protein and glucose concentrations, which indicates the activation of the processes in muscles. By the 7th day, the factor structure of activated lipolysis could be observed, whish is similar to acute stress. With the administration of DAGO, the structure of factors for all the periods of the study mainly included the correlated indicators of LPO in blood plasma and liver tissue, as well as lipid metabolism. Changes in the factor structure with the administration of DSLET and dynorphin A (1-13) were characterized by a greater divergence of the indicators included in the factor structure.

Conclusion

The obtained results confirm the literature data on the presence of phase changes in the liver after immobilization stress, depending on the stage of the stress reaction (Ivanov 1998). The data of the present study confirm the development of dystrophic changes in hepatocytes and impaired intrahepatic microcirculation. The study confirms the early onset of reparative processes in the liver after stress: after 39 hours, the number of binuclear cells and hepatocytes containing more than one nucleolus increases significantly in animals that were exposed to immobilization. Stress exposure leads to the development of a complex of structural changes in the liver, indicating the formation of an adaptive response. Moreover, it should be noted that this process is realized in the conditions of limited possibilities of its plastic provision, since the general direction of metabolic processes is characterized by predominating catabolism (Shkurupij 1989).

The development of dystrophic changes in hepatocytes is associated with the action of high concentrations of catecholamines (Klimov and Nikulcheva 1995), causing impaired intrahepatic microcirculation, hypoxia, and activation of lipid peroxidation.

The presented data confirm the fact that a degree of stress-induced disturbances in hepatocytes increases only as long as a certain strength of the stimulus is reached, its further increase is not accompanied by the worsening of the process manifestations.

Simulation of acute 6- or 12-hour stress was accompanied by a decrease in plasma protein levels as early as 39 hours after the exposure. It is assumed in this study that such a change is accounted for, first of all, by the activation of proteolysis in the initial period of the experiment. Impairment of the protein-synthetic function of the liver occurs later, on the 4th day after the exposure, when the concentration of albumin in the blood decreases. The chronic effect of the stress factor leads to a decrease in the content of albumin and protein in blood plasma. In this case, such changes are primarily associated with inhibition of the protein-synthetic function of the liver.

An increase in TC and NEFA contents in plasma in stress is a typical change, which is due to increased triglyceride mobilization and NEFA esterification in adipose tissue under the influence of adrenaline (Trilis et al. 2006). A similar effect is due to the activation of adenylate cyclase by catecholamines released under stress, which provides a shift of the metabolism from carbohydrates to lipids (Hulbert et al. 2005). An increase in TC in plasma is associated with the inhibition of the key enzyme of cholesterol catabolism – 7-alpha hydroxylase (Klimov 1992) by LPO products, which are accumulated during stress. Earlier it was shown that an increase of NEFA concentration in blood leads to increased formation of LDLs (Trilis et al. 2006).

An insignificant increase in the contents of TC and NEFA under the influence of swimming stress in comparison with immobilization stress is noteworthy. It is assumed in this study that this can be explained by the fact that intense exercise during swimming is accompanied by active consumption of lipolysis products, although the 2-hour swimming stress compared with a 6-hour immobilization stress may be characterized by a shorter and stronger effect of the stress factor.

As early as 39 hours after the exposure to a 3-hour immobilization stress or after intensive swimming, there is an increase in the formation of HDL, which indicates a high functional activity of hepatocytes. The trigger of HDL hyperproduction can be moderate activation of LPO, as it is known that HDLs have a pronounced antioxidant effect (Bueverov 2002).

On the contrary, a decrease in the concentration of HDLs in plasma may reflect the inhibition of the functional activity of hepatocytes under the influence of severe stress. The accumulation of LPO products has also a depressant effect on the formation of HDLs (Bueverov 2002).

Currently, there is no doubt about the leading role of impaired prooxidant-antioxidant balance and activation of lipid peroxidation in the development of stress-induced lesions of various organs (Lishmanov and Maslov 1994). Numerous literature data confirm the fact that exposure to an extreme irritant causes structural and functional abnormalities in the liver (Ivanov 1998). In particular, microcirculation abnormalities develop, which lead to hypoxia of hepatocytes and intensification of LPO processes with subsequent damage to mitochondria and necrosis of liver cells (Simonenkov and Fedorov 2008). On the basis of the results obtained, it can be concluded that the action of a moderately strong stressor stimulates SOD and catalase activities, and its long-term action, on the contrary, causes inhibition of enzyme activity, both in blood plasma and in liver tissue. It is known that the main function of SOD is the destruction of the superoxide an-

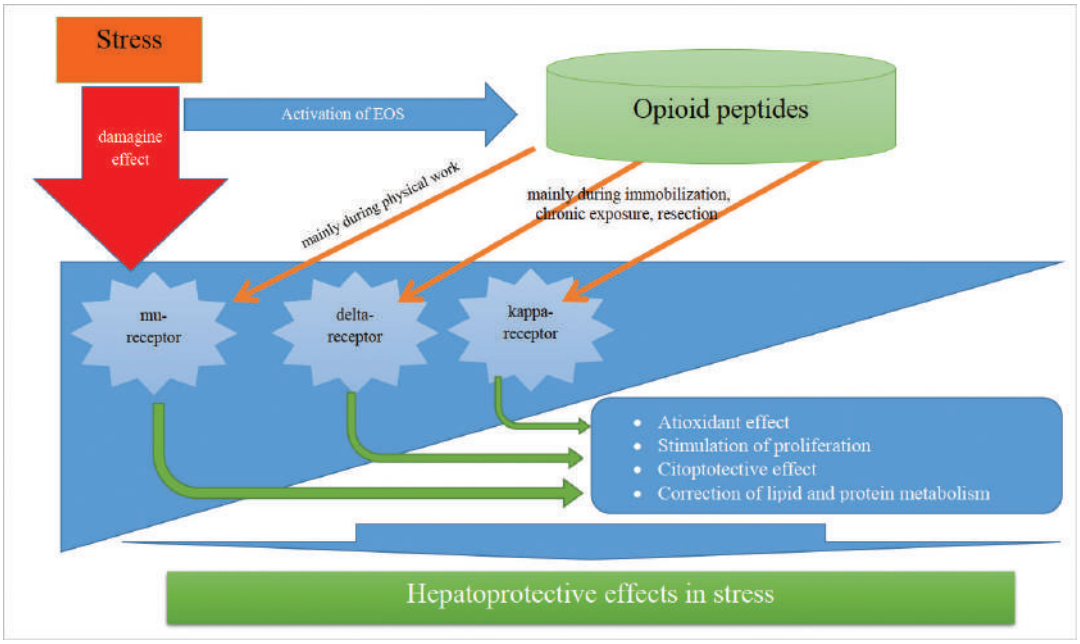

Figure 5. The mechanism of the hepatoprotective effect of OPs under stress.

ion radical, which is formed in excess when free radical oxidation is activated. The superoxide anion radical not only causes damage to cellular structures, but also interacts with hydrogen peroxide in the Haber-Weiss reaction, which results in the formation of one of the most reactive oxygen forms, the hydroxyl radical. In the experiments of the present study, an increase in SOD activity during short immobilization is an important adaptive factor that reduces the concentration of superoxide anion radical. A decrease in SOD activity in liver tissue during swimming stress indicates a drop in the potential of antioxidant systems, which counteracts the activation of free radical oxidation. A decrease in SOD activity in the liver tissue may be associated with the accumulation of oxidized metabolic products in the rats under conditions of intense physical exertion. A simultaneous increase in SOD activity in plasma can be explained by increased activity of this enzyme in other organs and tissues, which, among other things, secrete it into blood. The increased activity of catalase in the liver reflects the general direction of changes in the activity of antioxidant enzymes with a short action of an extraordinary factor.

A decrease in the activities of both SOD and catalase, both in plasma and in liver tissue in the course of simulated chronic stress, confirms the conclusion that the activity of antioxidant enzymes is inhibited during prolonged exposure to a stress factor. It was previously shown that the suppression of catalase activity is observed at the time of prolonged immobilization, which is due to the inhibitory effect of excessive concentrations of MDA and AGP, as well as possible structural changes in the enzyme molecule as a result of its glycation (Zor'kina et al. 1997). It is established that a low level of catalase enhances a toxic effect of lipid peroxidation products (Zborovskaja and Bannikova 1995). However, LPO cannot be considered solely as a mechanism of damage. At present, a large amount of information has been accumulated confirming the fact that free radicals are secondary messengers and, thus, are involved in the regulation of various functions of the body (Dubinina 2001).

The present study makes it possible to make a conclusion about the hepatoprotective effect of OPs during stress, which is manifested by a decrease in dystrophic and microcirculatory abnormalities in the liver tissue. A comparative study of the effect of selective OR agonists in equimolar doses on morphological and functional changes in the liver tissue during acute or chronic immobilization stress shows that the most pronounced hepatoprotective effect is observed when activating the dynorphinergic link of EOS. The high stress-protective activity of dynorphin A (1-13) in the liver tissue is explained, apparently, by the significant presence of kappa receptors in this organ, as well as the peculiarities of OP metabolism under the influence of specific and nonspecific peptidases (Panchenko et al. 1999). In the simulation of swimming stress, a selective mu-OR agonist DAGO had the strongest hepatoprotective effects. It is assumed in this study that these differences are explained by the peculiarities of metabolic processes during intensive swimming. Physican exercise, as known, is accompanied by the accumulation of non-oxidized products in the muscle tissue, which undergo further metabolic transformations in the liver. Apparently, the intense effect of non-oxidized products changes the sensitivity of different types of OR toward selective agonists, increasing the activity, first of all, of mu-OR.

This indicates the peculiarities of the implementation of the stress-protective effect of the components of the

stress-limiting system under various types of stress. It was established that the hepatoprotective effect of opioids was more pronounced in stress-susceptible animals, which were characterized by insufficiency of stress-limiting systems, when comparing the effects of OPs in animals of various typological groups. The mechanism of the hepatoprotective effect of OPs under stress includes the correction of the prooxidant-antioxidant balance, the stimulation of adaptation processes in the system of antioxidant enzymes, the cytoprotective effect and the correction of microcirculation abnormalities in the liver tissue under stress (Fig. 5). High catalase activity in the animals treated with dynorphin A (1–13) is noteworthy, since the prevention of excessive formation of hydrogen peroxide significantly reduces the toxic effect of LPO activation (Lishmanov and Maslov 1994). The high activity of SOD in the liver tissue in the rats treated by dynorphin A (1-13) is associated with a relatively low content of MDA, and, consequently, a decrease in its toxic effect on the enzyme.

Opioids have been shown to prevent stress-induced lipid metabolism disorders, and no specific effects of individual selective OR agonists have been detected.

Of interest is the fact established in the paper about the stimulating effect of chronic stress on liver regeneration in the initial period of this process.

In experiments with PHE simulation, it was shown that all the studied opioids stimulated the regeneration of liver tissue, and selective kappa-OR agonists dynorphin A (1-13) and mu-OR DAGO showed the most pronounced effect. It is assumed in this study that the stimulating effect of OPs on the regeneration of liver tissue after PHE may be explained by the activation of the formation of the necessary growth factors, in particular, the key component of the cascade, the hepatocyte growth factor. The action of opioids is accompanied either by an increase in the synthesis of hepatocyte growth factor in the liver tissue, or by its faster activation, which leads to an acceleration of the mitotic division of the remaining cells. DAGO has the most pronounced similar effect, which is why its stimulating effect was observed throughout all periods of the study. Dynorphin A (1-13) can activate the release of a transforming growth factor alpha more than other OPs, therefore its activating effect on the regeneration of the liver is most pronounced at the final stage (on the 7th day).

Thus, the findings of the present study reveal the mechanisms of participation of the endogenous opioid system in the prevention of liver damage in stress, and open up prospects for the use of pathogenetic therapy based on OP analogues in stress-induced liver damage, as well as other forms of its pathology.

Conflict of interests

The authors have no conflict of interest to declare.

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