DSLET and $ACTH_{4-10}$ Increase Mitotic Activity of Hepatocytes and Suppress Antibody Production

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The mitotic index of hepatocytes remained unchanged after 10 intraperitoneal injections of DSLET and $ACTH_{4-10}$ in doses of 0.5, 1.5, and 5 μg/kg, but increased after injection of these substances in doses of 50 and 150 μg/kg. DSLET in doses of 5, 50, and 150 μg/kg decreased the number of antibody-producing cells in the spleen. $ACTH_{4-10}$ possessed immunosuppressive activity not only in these doses, but also in a dose of 1.5 μg/kg. As differentiated from mitotic activity of hepatocytes, the degree of immunosuppression increased with increasing the dose of test peptides.

Key Words: *DSLET; $ACTH_{4-10}$; hepatocyte mitotic index; immune reactivity*

Recent studies showed that neuropeptides can modulate functional state of the liver. The number of various opioid receptors on liver cells is comparable with that in the brain [2]. Opioid peptides modulate bile excretion in the liver [8] and oxidative and carbohydrate metabolism in hepatocytes [7]. Under conditions of toxic hepatopathy transcranial electrostimulation recovers synthetic and detoxifying functions of the liver, inhibits cytolysis, and attenuates histological signs of fatty degeneration [7]. Naloxone blocks these changes, which indicates that they are realized via endorphins. $ACTH_{4-10}$ and Semax ($ACTH_{4-7}$ analogue) affect regeneration in several tissues: $ACTH_{4-10}$ stimulates proliferative processes in the cornea, while Semax improves wound healing [4,5]. The liver is not the immune organ. However, there is a close relationship between processes in the liver and immune system [1]. Functional activity of the liver modulates immune reactivity. It should be emphasized that the immune system affects regeneration of the liver. The effects of neuropeptides on proliferation and immunomodulatory activity of hepatocytes are poorly understood. Here we studied hepatoprotective and immunomodulatory activity of the selective δ-opioid receptor agonist DSLET and $ACTH_{4-10}$.

MATERIALS AND METHODS

Experiments were performed on CBA mice weighing 22-25 g. $ACTH_{4-10}$ was synthesized at the Institute of Molecular Genetics (Russian Academy of Sciences). DSLET was synthesized at the Research Center for Cardiology (Russian Academy of Medical Sciences). The peptides were injected intraperitoneally in doses of 0.5, 1.5, 5, 50, and 150 μg/kg (in 0.1 ml sterile isotonic NaCl, 10 times at 24-h intervals. The animals were killed by exsanguination under ether anesthesia 24 h after the last treatment. The liver was removed. Liver samples were fixed with 10% formalin in 0.1 M phosphate buffer (pH 7.2) and embedded in paraffin. Paraffin sections were stained with hematoxylin and eosin and subjected to morphological examination. The mitotic index of hepatocytes was calculated. The peptides were administered for 10 days before, during, and after immunization with sheep erythrocytes. The humoral immune response was evaluated by the number of antibody-producing cells in the spleen on day 5 after immunization [3]. The data are presented as $M \pm m$.

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TABLE 1. Effects of DSLET and $ACTH_{4-10}$ on Mitotic Index of Hepatocytes and Immunomodulatory Activity ($M \pm m$, n=9-10)

Peptide dose, μg/kg	DSLET		$ACTH_{4-10}$	
	mitotic index, ‰	antibody-producing cells, 10^3 per spleen	mitotic index, ‰	antibody-producing cells, 10^3 per spleen
Control (NaCl)	5.7±0.8	32.5±5.1	4.9±0.5	33.4±5.4
0.5	5.9±0.9	30.1±4.5	5.3±0.7	27.8±4.1
1.5	6.1±1.1	25.2±3.2	5.6±0.8	18.1±2.7*
5	6.5±1.3	19.7±2.8*	6.1±1.2	17.2±2.4*
50	9.6±1.5°	13.3±2.2°	8.7±1.3+	11.2±1.8+
150	9.8±1.6°	12.4±2.1°	9.2±1.6+	9.1±1.6°

Note. p<0.05: *compared to the control; +compared to the control and dose 0.5 μg/kg; °compared to the control and doses 0.5 and 1.5 μg/kg.

The significance of differences was estimated by Student's t test and Mann—Whitney U test.

RESULTS

The mitotic index of hepatocytes remained unchanged after injection of DSLET in doses of 0.5, 1.5, and 5 μg/kg, but considerably increased after administration of the peptide in doses of 50 and 150 μg/kg (Table 1). The effects of doses 50 and 150 μg/kg were similar. DSLET in doses of 0.5 and 1.5 μg/kg had no effect on the humoral immune response, but in higher doses the peptide produced an immunosuppressive effect (the number of antibody-producing cells decreased compared to that in animals receiving isotonic NaCl). Immunosuppressive activity of DSLET became more pronounced after increasing the dose. DSLET in a dose of 150 μg/kg produced maximum immunosuppressive effect. Immunosuppression produced by DSLIT in doses of 50 and 150 μg/kg was more pronounced compared to that observed after injection of 0.5 and 1.5 μg/kg peptide.

$ACTH_{4-10}$ in doses of 50 and 150 μg/kg increased the mitotic index of hepatocytes (similarly to DSLET). These changes were not observed after administration of the peptide in doses of 0.5, 1.5, and 5 μg/kg. $ACTH_{4-10}$ in doses of 1.5, 5, 50, and 150 μg/kg reduced immune reactivity. The degree of immunosuppression increased with increasing the dose of $ACTH_{4-10}$. Probably, immunosuppressive activity of $ACTH_{4-10}$ was higher than that of DSLET. The immunosuppressive effects were manifested after treatment with $ACTH_{4-10}$ and DSLET in doses of 1.5 and 5 μg/kg, respectively.

Published data show that the intensity of regenerative processes is low under conditions of immunodeficiency [1]. At the same time, DSLET and $ACTH_{4-10}$ suppress the humoral immune response and stimulate regeneration. Therefore, DSLET and $ACTH_{4-10}$ affect hepatocytes directly, rather than through the immune system. There are several mechanisms underlying suppression of immune reactions in the liver [1]. Humoral immunosuppressive factors secreted by the liver inhibit immune reactions not only in this organ, but also in extrahepatic tissues. They are secreted by hepatocytes due to initiation of mitotic reactions and suppress production of immune complexes causing injury in the liver. It cannot be excluded that these peptides produce a direct immunosuppressive effect on the immune system. DSLET *in vitro* inhibits proliferation of lymphocytes [3]. DSLET and $ACTH_{4-10}$ possess similar hepatotropic and immunomodulatory properties. These data suggest that the hepatotropic and immunomodulatory effects of the test peptides are realized via the same mechanisms.

Our results indicate that DSLET and $ACTH_{4-10}$ increase mitotic activity of hepatocytes and possess immunosuppressive activity. These peptides hold much promise for the therapy of liver diseases. DSLET and $ACTH_{4-10}$ activate regenerative processes in the liver and suppress possible autoimmune reactions.

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