DOI 10.1007/s10517-021-05072-z

Morphofunctional State of the Large Intestine in Rats under Conditions of Restraint Stress and Administration of Peptide $ACTH_{(4-7)}$-PGP (Semax)

M. V. Svishcheva[1], Ye. S. Mishina[1], O. A. Medvedeva[1], I. I. Bobyntsev[1], A. Y. Mukhina[1], P. V. Kalutskii[1], L. A. Andreeva[2], and N. F. Myasoedov[2]

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 170, No. 9, pp. 383-387, September, 2020
Original article submitted April 26, 2020

We studied the effect of intraperitoneal administration $ACTH_{(4-7)}$-PGP in doses of 5, 50, 150, and 450 μg/kg to Wistar male rats 12-15 min before modeling restraint stress on the morphofunctional state of the colon. In rats exposed to restraint stress, signs of atrophy and inflammatory reaction in the colon wall, changes in functional activity and number of mast cells, and increased serum level of corticosterone were observed. Administration of the peptide led to a decrease in corticosterone concentration, alleviated stress-induced pathomorphological changes, and promoted adaptation of the intestinal wall to stress. The positive effects of $ACTH_{(4-7)}$-PGP can be determined by multifunctional nature of the physiological and pharmacological effects of the neuropeptide.

Key Words: *Semax; restraint stress; colon; corticosterone*

Adaptation to stressful exposures involves all levels of biological organization in the human body, including the cellular and tissue levels [8,14]. The morphofunctional changes in the cardiovascular, nervous, respiratory, and endocrine systems under conditions of acute and chronic stress have been studied in detail [9,10,12,13]. However, the state of the colon during stress remains insufficiently studied. In addition, the search for drugs effectively correcting stress-induced changes without producing side effects is an urgent question.

Here we studied the effect of $ACTH_{(4-7)}$-PGP on the morphofunctional state of the colon under conditions of chronic restraint stress.

MATERIALS AND METHODS

The experiments were carried out on 60 male Wistar rats weighing 200-230 g, obtained from the SPF vivarium of the Federal Research Centre Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Sciences. The animals were quarantined in the vivarium of the Kursk State Medical University. The rats were kept at 22-24°C and 12/12 h light/dark regime and had free access to water and standard granular food. All studies were carried out in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental Purposes, the Directive 2010/63/EC of the European Parliament and the Council of the European Union (September 22, 2010) On the protection of animals used for scientific purposes, and in accordance with the decision Regional Ethics Committee of the Kursk State Medical University.

An analogue of hormone fragment $ACTH_{(4-7)}$-PGP (Met-Glu-His-Phe-Pro-Gly-Pro; active substance of Semax) lacking hormonal activity synthesized at the Institute for Molecular Genetics, Russian Academy of Sciences was used in the experiments. The peptide was dissolved in isotonic NaCl and injected intraperitoneally to male rats 12-15 min before modeling restrain stress in doses of 5, 50, 150, and 450 μg/kg (1 ml per

[1]Kursk State Medical University, Ministry of Health of the Russian Federation, Kursk, Russia, [2]Institute of Molecular Genetics, Russian Academy of Sciences, Moscow, Russia. ***Address for correspondence:*** bobig@mail.ru. I. I. Bobyntsev

0007-4888/20/1703-0384

1 kg body weight). Control stressed and intact animals were injected with equivalent volumes of saline at the same time intervals.

Chronic restraint stress was modeled by placing the rats into individual plastic boxes that corresponded to rat sizes daily for 2 h for 14 days [11]. Then, the animals were sacrificed by bleeding under anesthesia and the colon was sampled. The autopsy material was fixed in 10% neutral formalin. Paraffin sections were stained with hematoxylin and eosin. Microscopy and microphotography were carried out using an optical system consisting of a Leica CME microscope and a DCM-510 eyepiece camera at ×40, ×100, ×200, and ×400; the images were documented using FUTURE WINJOE software.

On histopathological preparations, the architectonics of the layers, the depth of the crypts, and the number of goblet cells were determined. The cell composition of the inflammatory infiltrate was also calculated; the cells were identified by karyological signs as neutrophils, lymphocytes, macrophages, and plasmacytes. The percentage of the cells and the total number of cells were assessed in 10 fields of view on an area of 502.08 μ^2. Functional activity of mast cells (MC) was determined by counting different types of MC based on the Linder's classification.

Corticosterone concentration in blood serum was measured by ELISA using Corticosterone ELISA Kit ADI-900-097 (Enzo).

Statistical data processing was performed using the algorithms of the Statistica Trial software. After checking the normality of data distribution using the Kolmogorov—Smirnov test, the parametric Student's *t* test was applied. The results were considered significant at $p \leq 0.05$.

RESULTS

Administration of saline to intact animals was not accompanied by morphological and functional changes in the colon. The colon had normal 4-layer structure. Crypts had a slightly bullous shape; their depth was 174.9 μ. Goblet cells were strictly oriented and weakly oxyphilic; their number was 67.2 per standard area (Fig. 1, *a*). Among MC, type 2 and 3 predominated (5.9 and 5.5, respectively; Table 1).

In rats subjected to chronic restraint stress and receiving saline, dyscirculatory and degenerative processes developed in the colon wall. Zones of surface necrosis with epithelium destruction were revealed. The depth of the crypts sharply decreased by 18.6% ($p \leq 0.01$). In the lamina propria, pronounced lymphocytic infiltration with admixture of segmented leukocytes and signs of interstitial edema were observed (Fig. 1, *b*). The number of goblet cells per unit area was significantly reduced by 12.9% ($p \leq 0.01$). The cellular composition was mainly presented by lymphocytes (36.9), macrophages (12.5), and neutrophils (7.3). During restraint stress, patchy follicular accumulation and active proliferation of inflammatory cells were seen in the entire thickness of the colon wall. Blood corticosterone concentration in stressed rats was 58.9% higher than in unstressed control ($p \leq 0.01$, Table 1).

Administration of $ACTH_{(4-7)}$-PGP in a dose of 5 mg/kg to stressed animals did not restore histoarchitectonics of the colon. Desquamation of the surface epithelium and edema of the submucosa and lamina propria were still observed. The crypts were smoothened and their depth did not significantly change; the glands had signs of degeneration (Fig. 2, *a*). The number of goblet cells was close to that observed in stressed animals (61.0; Table 1). Morphometric revealed prevalence of inflammatory cells: lymphocytes and neutrophils. The serum level of corticosterone decreased by 28.6% ($p \leq 0.01$).

Administration of $ACTH_{(4-7)}$-PGP in a dose of 50 μg/kg reduced the signs of inflammation in the colon tissue and a tendency of recovery of its histoarchitectonics was noted. Focal smoothing of the surface relief was revealed. Crypts were deepened compared to the stress control, the depth of which increased by 5.2% ($p \leq 0.01$). Insignificant edema remained in the under-

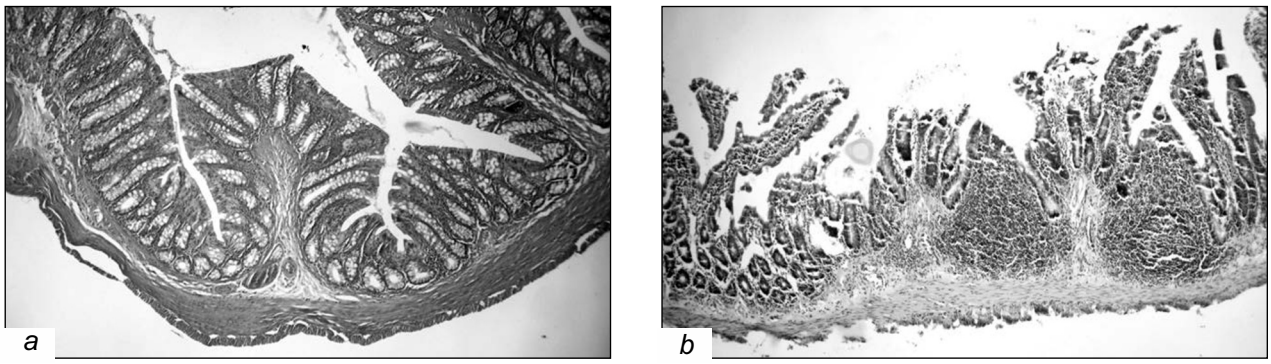

Fig. 1. Micrograph of the colon wall in group of intact control (*a*) and stressed control (*b*). Hematoxylin and eosin staining, ×40.

TABLE 1. Morphometric Parameters of the Colon and Corticosterone Levels in Rats under Conditions of Restraint Stress and Treatment with $ACTH_{(4-7)}$-PGP (absolute values; n=10; $M±m$)

Parameter	Intact control	Stressed animals				
		control	administration of Semax in a dose of			
			5 μg/kg	50 μg/kg	150 μg/kg	450 μg/kg
Crypt depth	174.9±0.78	142.4±1.93**	143.6±3.2	149.7±1.62++	151.2±1.41++	157.1±3.53++
Goblet cells	67.2±1.77	58.5±1.12**	61±2.39	62.2±1.21+	63.66±1.63+	75±1.38++
MC type 1	5±0.32	19.6±0.48**	22.7±0.83+	11.2±0.5++	7.7±0.37++	8.8±0.59++
MC type 2	5.9±0.21	14.5±0.51**	13.9±0.64	10.7±0.46++	7.9±0.42++	8±0.44++
MC type 3	5.5±0.33	17.1±0.62**	15.6±0.59	14.8±0.61+	11.6±0.5++	8.6±0.56++
MC type 4	4.9±0.26	13.4±0.47**	13.4±0.64	15.3±0.43++	11.2±0.51++	10.3±0.72++
Neutrophils	1.6±0.14	7.3±0.52**	6.1±0.59	3.6±0.29++	3.3±0.2++	3.3±0.27++
Lymphocytes	19.4±0.53	36.9±0.82**	33.1±0.99+	24.6±0.78++	24.7±0.91++	24.1±0.81++
Macrophages	4±0.34	12.5±0.72**	10.7±0.54	14.6±0.49+	15.5±0.77+	17.1±0.65++
Plasmacytes	10.2±0.37	14.4±0.81**	12.3±0.56+	12±0.56+	12.8±0.93	18.3±1.16++
Corticosterone	1360.9±57.79	2162.5±170.77**	1543.7±90.92++	1423.3±31.9++	1580.6±95.57++	1466.9±21.42++

Note. **p≤0.01 in comparison with intact control, +p≤0.05, ++p≤0.01 in comparison with stressed control

lying departments. There is enhanced proliferation of the cervical epithelium cells (Fig. 2, *b*). The number of goblet cells increased significantly by 6.3% (p≤0.05). There was sclerosis in the submucosa and slight lymphocytic infiltration: the number of lymphocytes was 24.6 (Table 1).

In animals treated with the peptide at a dose of 150 μg/kg, signs of restoration of rat colon architectonics were detected. Crypts were deeper by 6.3% than in stressed control rats and dilated by the foveolar type (p≤0.01). The number of goblet cells increased by 8.9% in comparison with stressed control (p≤0.05). No signs of edema were detected. Insignificant lymphocytic infiltration and sclerosis were observed in the lamina propria of the submucosa and mucosa. Enhanced proliferation of the cervical epithelium persisted (Fig. 2, *c*). The number of MC of all 4 types (according to Linder's classification) was significantly reduced in comparison with stressed control.

Significant morphological changes in the colon after administration of $ACTH_{(4-7)}$-PGP in doses of 50 and 150 μg/kg corresponded to a significant decrease in serum corticosterone concentration in experimental animals by 34.2 and 27%, respectively (p≤0.01).

After administration of $ACTH_{(4-7)}$-PGP in a dose of 450 μg/kg, no atrophic and degenerative changes in the colon mucosa were detected. However, foci with a smoothed mucous membrane and zones of adenomatous proliferative activity were detected against the background of weak inflammatory reaction, the depth of crypts did not reach the values observed in intact animals. An increase in the number of both individual goblet cells and the total number of glands was observed. The height of the cubic epithelium increased (Fig. 2, *d*). Morphometry revealed significant predominance of monocytic cells. The number of plasmacytes increased by 27.1% in comparison with the stressed control group (p≤0.01). Diffuse growth and thickening of collagen fibers and sclerosis led to an increase in the colon wall thickness. The corticosterone level decreased by 32.2% (p≤0.01, Table 1).

Our findings suggest that the main manifestations of the stress response in the colon were inflammation, atrophy, and increase in the number and activity of MC. Treatment with $ACTH_{(4-7)}$-PGP increased the rate of adaptation and tissue reorganization. These effects of the peptide can be associated with broad range of its pharmacological and physiological effects [3-5]. The effect of $ACTH_{(4-7)}$-PGP on the morphofunctional state of the colon can be determined by its immunomodulating effect, because the drug affects both humoral and cellular reactions [6]. It was found that preliminary administration of the neuropeptide to stressed rats inhibited macrophage-mediated and proliferative processes and reduced destructive processes in the spleen [1]. Semax has a direct cytotoxic effect on atypical cells. The mechanisms of the stress-limiting effect of the peptide can also be influenced by sympathetic innervation and microcirculation [2,7].

Thus, administration of $ACTH_{(4-7)}$ -PGP leveled stress-induced pathomorphological changes in the colon by increasing the rate of adaptation, reducing the number of inflammatory cells, and changes in MC activity. The most pronounced effects were observed

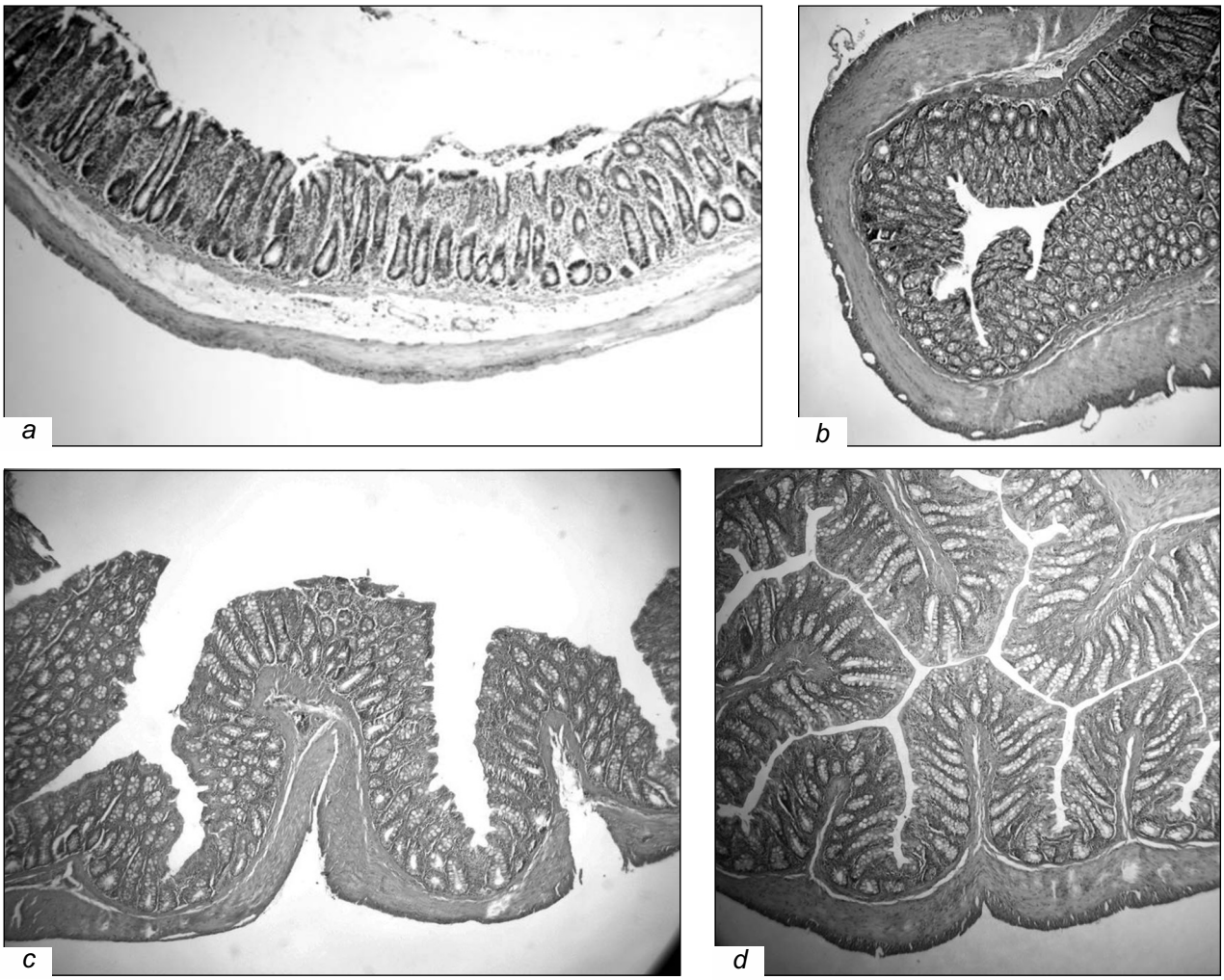

Fig. 2. Micrograph of the colon wall in rats treated with Semax in doses of 5 (*a*), 50 (*b*), 150 (*c*), and 450 μg/kg (*d*). Hematoxylin and eosin staining, ×40.

after injection of ACTH-4-7-PGP in doses of 50 and 150 μg/kg.

REFERENCES

1. Bakhmet AA, Koplik EV. Antistress effect of Semax in the course of recovery of spleen lymphoid structures after the stress in rats with different behavioral activity. Bull. Exp. Biol. Med. 2012;153(5):661-663. doi: 10.1007/s10517-012-1792-7
2. Gavrilova SA, Markov MA, Berdalin AB, Kurenkova AD, Koshelev VB. Changes in Sympathetic Innervation of the Heart in Rats with Experimental Myocardial Infarction. Effect of Semax. Bull. Exp. Biol. Med. 2017;163(5):617-619. doi: 10.1007/s10517-017-3862-3
3. Levitskaya NG, Glazova NYu, Sebentsova EA, Manchenko DM, Vilenskii DA, Andreeva LA, Kamenskii AA, Myasoedov NF. Evaluation of the spectrum of physiological activity of ACTH4-10 analog of heptapeptide Semax. Neirokhimiya. 2008;25(1):111-118. Russian.
4. Manchenko DM, Glazova NYu, Levitskaya NG, Andreeva LA, Kamensky AA, Myasoedov NF. Nootropic and analgesic effects of Semax following different routes of administration. Ross. Fiziol. Zh. 2010;96(10):1014-1023. Russian.
5. Romanova GA, Silachev DN, Shakova FM, Kvashennikova YN, Viktorov IV, Shram SI, Myasoedov NF. Neuroprotective and antiamnesic effects of Semax during experimental ischemic infarction of the cerebral cortex. Bull. Exp. Biol. Med. 2006;142(6):663-666. doi: 10.1007/s10517-006-0445-0
6. Samotrueva MA, Yasenyavskaya AL, Murtalieva VK, Bashkina OA, Myasoedov NF, Andreeva LA, Karaulov AV. Experimental Substantiation of Application of Semax as a Modulator of Immune Reaction on the Model of "Social" Stress. Bull. Exp. Biol. Med. 2019;166(6):754-758. doi: 10.1007/s10517-019-04434-y
7. Fadyukova OE, Tyurina AY, Lugovtsov AE, Priezzhev AV, Koshelev VB, Andreeva LA, Myasoedov NF. Semax increases erythrocyte deformability in the shearing blood stream in intact rats and rats with cerebral ischemia. Dokl. Biol. Sci. 2011;439(1):208-211. Russian.
8. Choi DC, Nguyen MM, Tamashiro KL, Ma LY, Sakai RR, Herman JP. Chronic social stress in the visible burrow system modulates stress-related gene expression in the bed nucleus of the stria terminalis. Physiol. Behav. 2006;89(3):301-310.

9. Hernandez ME, Martinez-Mota L, Salinas C, Marquez-Velasco R, Hernandez-Chan NG, Morales-Montor J, Pérez-Tapia M, Streber ML, Granados-Camacho I, Becerril E, Javier BH, Pavón L. Chronic stress induces structural alterations in splenic lymphoid tissue that are associated with changes in corticosterone levels in Wistar-Kyoto rats. Biomed. Res. Int. 2013;2013:868742. doi: 10.1155/2013/868742
10. Khandve B, Gujar V, Bokariya P, Tarnekar A, Shende M. Deranged spermatogenesis of adult Swiss Albino mice as effect of immobilization stress — histological study. Iosr. J. Pharm. 2013;3(2):7-10.
11. Kim MH, Leem YH. Chronic exercise improves repeated restraint stress-induced anxiety and depression through 5HT1A receptor and cAMP signaling in hippocampus. J. Exerc. Nutrition Biochem. 2014;18(1):97-104.
12. Popolo A, Autore G, Pinto A, Marzocco S. Oxidative stress in patients with cardiovascular disease and chronic renal failure. Free Radic. Res. 2013;47(5):346-356.
13. Troudi A, Bouaziz H, Soudani N, Ben Amara I, Boudawara T, Touzani H, Lyoussi B, Zeghal N. Neurotoxicity and oxidative stress induced by gibberellic acid in rats during late pregnancy and early postnatal periods: Biochemical and histological changes. Exp. Toxicol. Pathol. 2020;64(6):583-590.
14. Ulrich-Lai YM, Figueiredo HF, Ostrander MM, Choi DC, Engeland WC, Herman JP. Chronic stress induces adrenal hyperplasia and hypertrophy in a subregion-specific manner. Am. J. Physiol. Endocrinol. Metab. 2006;291(5):E965-E973.