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ROLE OF PERIPHERAL DOPAMINERGIC SYSTEM IN THE PATHOGENESIS OF EXPERIMENTAL COLITIS IN RATS

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Dopamine (DA) is produced and released by immune cells. Recent data pointed to DA as a key mediator between the nervous and immune systems. In the present study we tested the hypothesis that peripheral dopaminergic system plays a negative role in ulcerative colitis pathogenesis via the effect on activity of peripheral blood phagocytes. The study was conducted on male Wistar rats (170-200 g). The peripheral dopaminergic system was destroyed by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) injection (20 mg/kg, s.c., 4 times every 2 h). Colitis was induced by 0.1 ml 6% iodoacetamide enema. Rats were subjected to autopsy on the 18th day. We found that MPTP-treated rats had decreased levels of tyrosine hydroxylase, rate-limiting enzyme of DA synthesis, in colon but not in brain. The number and activity of colonic and peripheral blood granulocytes did not significantly differ in saline- and MPTP-treated rats with colitis. The decreased ROS production by monocytes; increased 1.8-fold the number of CD69 (an early activation marker) positive monocytes and 6-fold intensity of CD69 surface expression were observed in MPTP-treated rats vs. saline-treated rats during colitis. The CD14 (the endotoxin coreceptor of phagocytes) surface expression was 2-fold increased in MPTP-treated rats without colitis, but significantly decreased in both saline- and MPTP-treated rats with colitis. We showed for the first time that the destruction of peripheral dopaminergic neurons leads to the improvement of morphological signs of experimental colitis, which might be through the regulatory effect of dopaminergic system on monocytes phenotype and their respiratory burst activity.

K e y w o r d s: dopaminergic system, ulcerative colitis in rats, granulocytes, monocytes, CD69, CD14.

Dopamine (DA) is a neurotransmitter, which regulates various processes such as cognition, locomotion, hormone secretion and affects intestinal motility. Recent data indicates that DA is involved in immune responses in autoimmune diseases as well as in neurodegenerative disorders and sepsis [1].

It is known that about 40% of blood DA has peripheral origin, including gastrointestinal tract (GI) [2]. Various studies on sympactomized animals showed the presence of tyrosine hydroxylase (TH) in non-neuronal cells (epithelial, muscle, endothelial and leukocytes). Various pharmacological studies and studies on knockout mice also determined the existence of peripheral dopaminergic system [2, 3].

D1-D5 dopamine receptors were found throughout different parts of GI tract with mainly D2 and D3 receptors in the colon [4]. It was previously shown that D2 and D3 receptor agonists are able to suppress mast cell degranulation and production of pro-inflammatory mediators *in vitro* [5, 6]. D2 receptor activation on endothelial cells suppressed VEGF-induced increase of vascular permeability, reduced edema and provided vessel stabilization on cancer models [7-9] and during the ovarian hyperstimulation syndrome [10].

The decreased level of L-3,4-dihydroxyphenylalanine (L-DOPA) and DA were determined in colon mucosa of patients with inflammatory bowel disease (IBD), which combined both ulcerative coli-

tis and Crohn's disease, [11] and during experimental colitis in rats [12]. Moreover, D2R TaqIA polymorphism, responsible for the decrease in receptor density, is associated with refractory Crohn's disease [13].

Previously Tolstanova et al. [14] showed that D2 receptor expression in the colon was altered in patients with IBD and during experimental colitis in rats. D2 receptor activation accelerated the healing of lesions during experimental colitis through the downregulation of vascular permeability and, as a result, the reduction of inflammation. Two D2 receptor agonists (quinpirole and cabergoline) were used in the above study; both of them can cross blood brain barrier; hence, they act on the periphery and in the central nervous system. Thus, we could exclude the role of neither central nor peripheral dopaminergic system in the beneficial effect of D2 agonists on the colonic lesion. Moreover, the data on duodenal ulceration indicated that dopamine-related drugs affect experimental duodenal ulcers both by peripheral and central actions [15-17]. Furthermore the importance of central dopamine in intestinal mucosal integrity was confirmed by Ray et al. [16]. These authors showed that microinjection of DA or D2 agonist bromocriptine in amygdala dose-dependently attenuated stress-induced gastric ulcer formation in rats.

In our pilot study, we found that simultaneous activation of central and inhibition of peripheral D2 dopamine receptors had additive positive effect on the prevention of increased colonic vascular permeability during experimental colitis. These data further confirmed the need for a more extensive study to understand the role of dopaminergic system in ulcerative colitis pathogenesis. The aim of the present research was to test the hypothesis that peripheral dopaminergic system plays the negative role in ulcerative colitis pathogenesis via the effect on activity of peripheral blood phagocytes.

Materials and Methods

Animals. Male Wistar rats (170-200 g, n = 25) were housed under standard vivarium conditions. All animals had unlimited access to tap water and Purina chow. These studies were approved by Bioethical Committee of "Institute of Biology and Medicine", Taras Shevchenko National University of Kyiv (Kyiv, Ukraine), protocol No 1 from 20.02.2017.

MPTP-induced model of peripheral dopaminergic system destruction. To investigate the role of the peripheral dopaminergic system in the development of ulcerative colitis in rats, MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) model was used (Sigma, USA) [18]. One week prior to the beginning of the experiment rats were settled in separate cages for adaptation and catecholamine level stabilization. Animals were divided into 4 groups: group I (control) – on the 1st day rats were given 0.1 ml/rat saline (4 times every 2 h, s.c.), on the 7th day – 0.1 ml 1% methylcellulose rectally (n = 3); group II (MPTP) – on the 1st day rats were given 20 mg/kg MPTP (4 times every 2 h, s.c.) on the 7th – 0,1 ml methylcellulose rectally (n = 3); group III (IA) – on the 1st day rats were given 0.1 ml/rat saline (4 times every 2 h, s.c.), on the 7th day – 0.1 ml 6%-iodoacetamide, rectally (n = 3); group IV (MPTP+IA) – on the 1st day rats were given 20 mg/kg MPTP (4 times every 2 h, s.c.) on the 7th – 0.1 ml 6%-iodoacetamide rectally (n = 3). On the 18th day of the experiment (in 10 days after IA enema) animals were subjected to an autopsy by decapitation. Whole blood was collected to heparinised tubes for further cells isolation. Seven cm of colon tissue and brain were removed from rats and preserved in liquid nitrogen.

At autopsy macroscopic analysis of colonic lesions was performed by measurement of colitis score (0-3), colon wet weight (mg/100 g), loss of rugae (mm^2), dilatation (mm), lesioned area (mm^2). Disease activity index (DAI) was evaluated on 3rd and 7th days after IA enema, by summing up loss of body weight, diarrhea, lethargy as described previously [14]. Colon mucosa was scraped and frozen in liquid nitrogen for the further Western blot analysis.

Western blotting. At autopsy, the removed colon was cut along anti-mesenteric side and thoroughly rinsed in cold PBS. The colon was gently wiped by paper towel and flat by mucosa side up on ice. Using metal spatula we gently scraped mucosa from the muscular layer. Brain tissue, which consisted of substantia nigra part, was removed at autopsy. Total protein loads (100 μg of total proteins) extracted from colonic mucosa and brain tissue in a lysis buffer containing protease and phosphatase inhibitors were processed routinely for Western blot as described previously [19]. The primary antibodies against tyrosine hydroxylase (TH) (1 : 500) and β-actin (1 : 500) (Santa Cruz Biotechnology Inc., German) were used to determine for the protein expression levels in the rat colon mucosa and brain tissue with further incubation with anti-rabbit (anti-TH) and anti-mouse (anti-β-actin) secondary anti-

bodies (1 : 2500), conjugated with horseradish peroxidase. Visualization of the results was performed with ECL-reagent. Results were analysed using Phoretix1D software. Protein levels were determined by the number of fluorescent signal units using units of β-actin fluorescent signal for the standardization of initiate quantity of protein. Alterations in protein expression were counted by the difference of fluorescent signal units of the experimental group compare to the control group. Each Western blot analysis was repeated at least twice.

Myeloperoxidase (MPO) activity assay. Samples were homogenized with liquid nitrogen till the powder was formed. Then 1 ml HTAB buffer was added to homogenized sample. One ml of homogenate suspension was transferred into microtubes. Microtubes were subjected to 3 cycles: 1 min in liquid nitrogen, 10 min in water bath at 37 °C. Then the samples were sonicated for 10 s with ultrasound disintegrator with outcoming current – 0.5 A. After sonication the samples were centrifuged for 15 min (14 000 rpm, T = 4 °C). MPO solution in HTAB (Sigma-Aldrich) in concentrations 0.5 U/ml, 0.25 U/ml, 0.125 U/ml, 0.06 U/ml, 0.03 U/ml, 0.015 U/ml were used as standard. In 96-well plate standard solutions with different concentration, 50 µl each, were put. Fourteen µl of sample supernatant, received after centrifugation, were put in cells. In all cells 200 µl of reaction buffer (6.1 ml H_2O_2 solution, 4,1 ml ODHC solution and 4.4 ml phosphate buffer (pH = 6) were added. The density of samples was measured after 5-10 min at 450 nm wavelength spectrophotometrically (Bio-Rad, USA). MPO activity was calculated for g of tissue. Data was presented as MPO activity – U/g.

Intracellular reactive oxygen species (ROS) assay. ROS levels were measured using 2′7′-dichlorodihydro-fluorescein diacetate (H2DCFDA, Invitrogen) as previously described [20]. Briefly, heparinized whole blood cells were incubated with PBS containing 10 µM carboxy–H2DCFDA for 30 min at 37 °C to measure ROS production by peripheral blood monocytes and granulocytes. A short recovery time was allowed for the cellular esterases to hydrolyze the acetoxymethyl ester or acetate groups and render the dye responsive to oxidation. Erythrocytes were lysed with lysis buffer. The cells were then transferred to polystyrene tubes with cell–strainer caps (Falcon, Becton Dickinson, USA) and analysed with flow cytometry (excitation: 488 nm, emission: 525 nm). Only living cells, gated according to scatter parameters, were used for the analysis. Results were presented as mean fluorescence per cell [21, 22].

Phagocytosis assay. The flow cytometry phagocytosis assay was performed as previously described [20]. Briefly, FITC-labeled heat-inactivated *Staphylococcus aureus* Cowan I bacteria (collection of the Department of Microbiology and General Immunology of Taras Shevchenko National University of Kyiv) at the concentration of 1×10^7 cells/ml in the volume of 5 µl were added to heparinized whole blood. All samples were incubated at 37 °C for 30 min. At the end of the assay, phagocytosis was arrested by adding the cold stop solution (PBS with 0.02% EDTA and 0.04% paraformaldehyde). Erythrocytes were lysed with lysis buffer. Fluorescence of phagocytes with ingested bacteria was determined by flow cytometry. The results were registered as the percentage of cells emitting fluorescence after a defined culture period (phagocytosis percentage) and as phagocytosis index that representing the mean fluorescence per one phagocytic cell (ingested bacteria by one cell).

Immunofluorescence labelling. Phycoerythrin-conjugated anti-CD69 antibodies and FITC-labeled anti-CD14 antibodies (Becton Dickinson, Farmingen, USA) were used to determine the relative amount (percentage) of CD69+ and CD14+ cells (monocytes and granulocytes) and an intensity of CD69 and CD14 surface expression (mean fluorescence per cell) among circulating phagocytes. The rabbit anti-rat antibodies were added (5µl) in heparinized whole blood samples (50 µl) after erythrocyte lysis. The cells were incubated for 25 min at room temperature. Samples were analyzed by FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA). The data were analyzed using CELLQuest software (BD; Franklin Lakes, NJ, USA).

Statistical analysis. Statistical analysis of the results was performed using Statistica 8.0 software. Shapiro-Wilque criteria were applied to each sample. Mean and standard deviation were calculated for each of them. The significance of received results between groups was evaluated by Student's *t*-test. $P < 0.05$ was considered as statistically significant.

Results and Discussion

DA synthesis depends on the rate of conversion of amino acid tyrosine into the immediate precursor of dopamine L-DOPA by TH [23]. Studies on sympathectomized animals along with detection of non-neuronal TH-positive cells and measuring levels

of DA metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) revealed that DA activity on the periphery is far beyond simple precursor of norepinephrine/epinephrine synthesis [24].

In order to destroy peripheral dopaminergic system, rats were given subcutaneously neurotoxin MPTP which used to induce experimental model of Parkinson's disease. According to literature, dose of MPTP 4 × 20 mg/kg did not produce measurable changes in central dopaminergic neurons [25] but reduced the number of enteric TH-positive neurons as well as DA concentration in the intestine vs. the esophagus and stomach [26].

We showed that TH levels were decreased in colon, but remained unchanged in brain tissue of MPTP-treated rats (Fig. 1, *A, B*), which confirms the destruction of peripheral dopaminergic neurons. It is known that not only dopaminergic, but also noradrenergic neurons are destroyed during parkinsonism. While most of the dopaminergic neurons are destroyed, compensatory mechanism of TH production, maintained by noradrenergic neurons takes place. This helps to postpone the decrease of dopamine levels in brain and disease progression; with time of disease development, noradrenergic neurons are also destroyed [27].

To check the role of peripheral dopaminergic system in pathogenesis of IBD, saline and MPTP-treated rats were injected with IA-enema in order to induce experimental colitis. We found that MPTP-treated rats had more profound clinical signs of colitis vs saline-treated rats after IA enema (Fig. 2, *A*). While, macroscopic appearance of colitis was significantly better in MPTP vs. saline-treated rats (Fig. 2, *B-D*). We didn't observe any clinical as well as colonic macroscopic changes in MPTP vs. saline-treated rats after MC enema (Fig. 2, *B-D*). So, the destruction of dopaminergic neurons on periphery leads to the improvement of morphological signs of colitis in rats. Observed by us worse clinical signs of disease (e.g. lethargy, loss of body weight) in MPTP-treated rats allowed to speculate on the positive role of central dopaminergic system in IBD pathogenesis. Further study needs to confirm this assumption.

The exact cause of IBD has yet not been fully elucidated, although accessible data suggests that IBD results from adverse interactions between susceptibility genes, microbiome, the environment, and the immune system which can cause an excessive and abnormal immune response against host microbiome in genetically susceptible individuals [28]. Most recently described IBD susceptibility genes are linked to host immune system including epithelial barrier function, host defence mechanisms against pathogens as well as autophagy, innate and adaptive immune response [29]. IBD is characterized by up-regulation of proinflammatory cytokines (TNF-α, interleukin (IL)-6, IL-13, IL-17, IL-18, and IL-21) and decrease of anti-inflammatory cytokines (IL-10, IL-11, and transforming growth factor β) [30].

Emerging evidence pointed to DA is a key transmitter between the nervous system and the im-

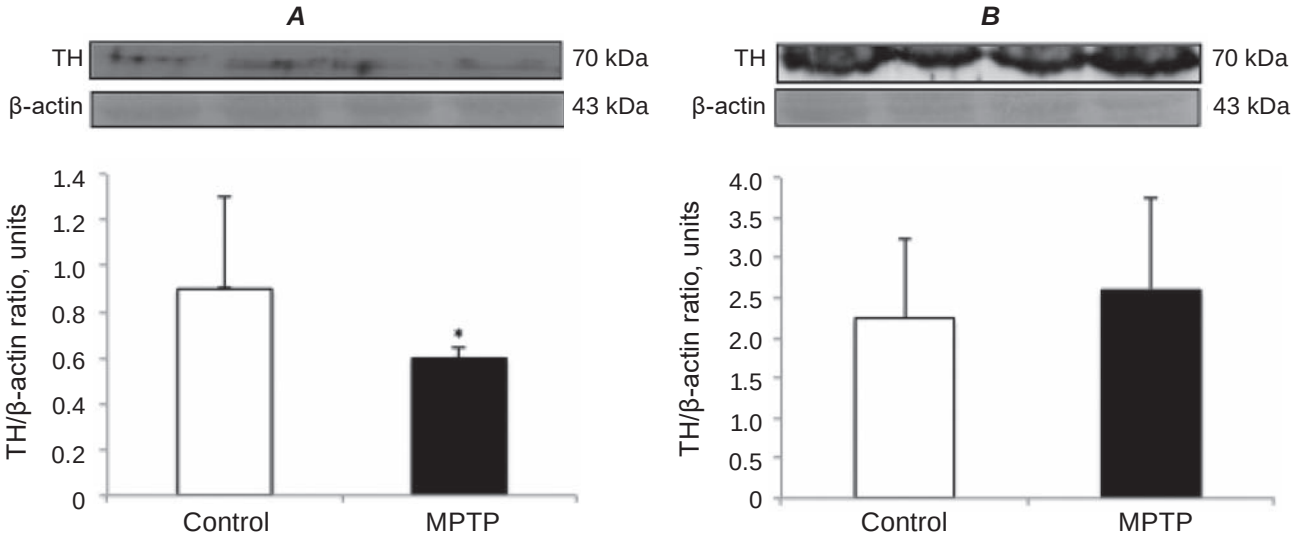

*Fig. 1. Levels of tyrosine hydroxylase (TH) in rat colonic mucosa (**A**) and brain (**B**) after treatment with neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, 4×20 mg/kg, s.c.). *P < 0.05 vs. control group*

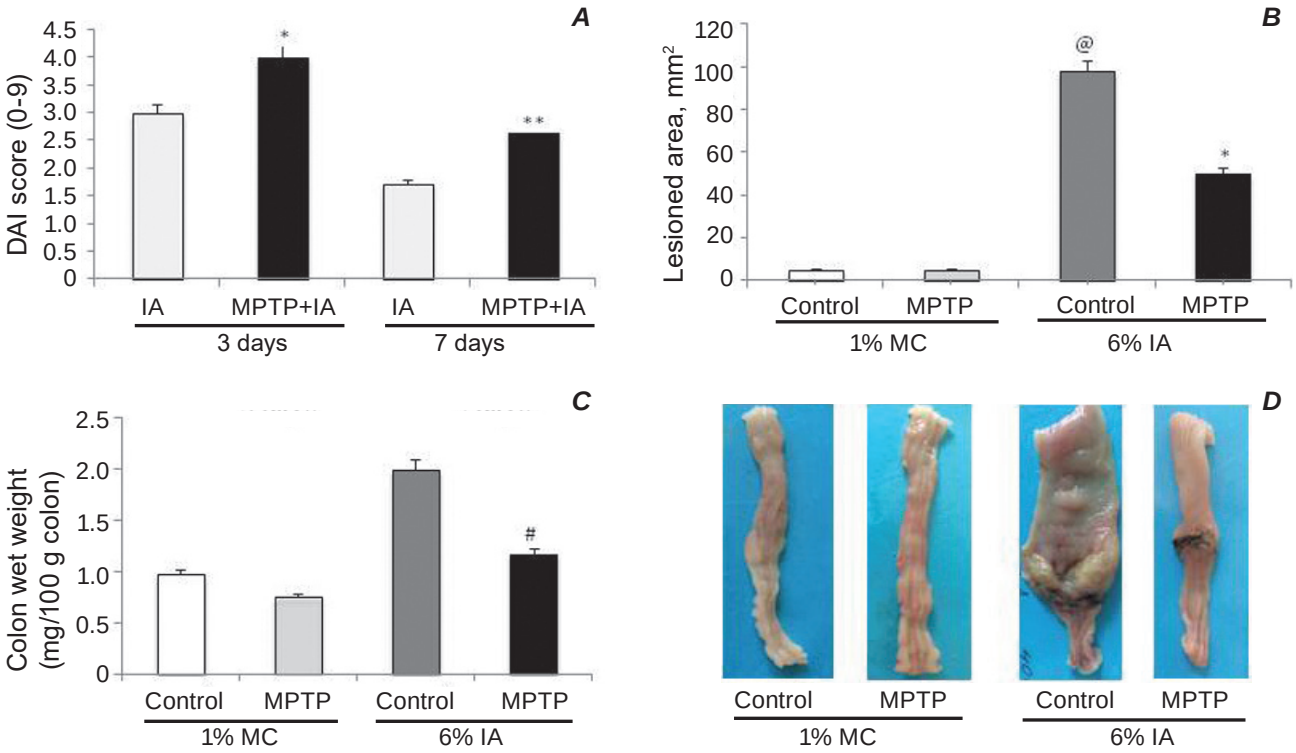

Fig. 2. Clinical and macroscopic features of iodoacetamide-induced colitis in saline and MPTP-treated rats. ***A*** *– disease activity index;* ***B*** *– the size of colonic lesions;* ***C*** *– colonic wet weight mg/100 g colon;* ***D*** *– macroscopic appearance of colonic ulceration 10 days after iodoacetamide administration (M ± SD, @ P < 0.05 – vs. control group. * P < 0.05, vs. the IA group (3 days of colitis), ** P < 0.05, vs. IA group (7 days of colitis). # P < 0.05 vs. IA group. Changes of macroscopic and clinical features of UC development in control rats and rats injected with MPTP (20 mg/kg) (B, C)*

mune system as well as a mediator produced and released by immune cells themselves [31]. Several studies now indicate the presence of DA D1, D2, D3, D4 and D5 receptors in normal human leukocytes. Among the leukocyte subpopulations, T lymphocytes, monocytes have low, neutrophils, eosinophils have moderate and B, NK cells have high and more consistent expression of dopamine receptors. In addition, DA D1 receptors are present in human dendritic cells. Dopamine uptake system has also been identified in the lymphocytes [32].

To check the underline mechanism of improved morphologic features of colitis in MPTP- vs. saline-treated rats, we examined key parameters of immunological response during IBD.

Infiltrating neutrophils are the first line of host defense against a wide range of infectious pathogens exerting their role in host defense through the secretion of cytokines, proteases, ROS generation and neutrophil extracellular traps formation [33]. Functional studies performed in *in vitro* conditions, reported inhibitory effects of dopamine on fMLP-stimulated superoxide anion production by human neutrophils [34]. Dopamine has also been reported to attenuate CD11b/CD18 expression in neutrophils, with consequently diminished ability of human neutrophil adherence to the endothelium, as well as a decrease in the production of ROS and superoxide anions, cell migration and phagocytic activity [35].

In our study, the MPO levels in colonic mucosa, the classical marker of infiltrating neutrophils, did not differ in MPTP and saline-treated rats with IA-induced colitis (data not shown). In peripheral blood, development of IA-induced colitis in both MPTP and saline-treated rats was associated with significant increase of granulocytes in phagocytosis (mostly neutrophils) (Fig. 3, *B*) and granulocytes respiratory burst (Fig. 3, *A*) in comparison to control group (no colitis). While in MPTP-treated rats without colitis, the number of granulocytes in phagocytosis was significantly decreased (Fig. 3, *B*).

To further analyze the profile of granulocytes we checked the number of CD69 positive granulocytes and intensity of CD69 surface expression.

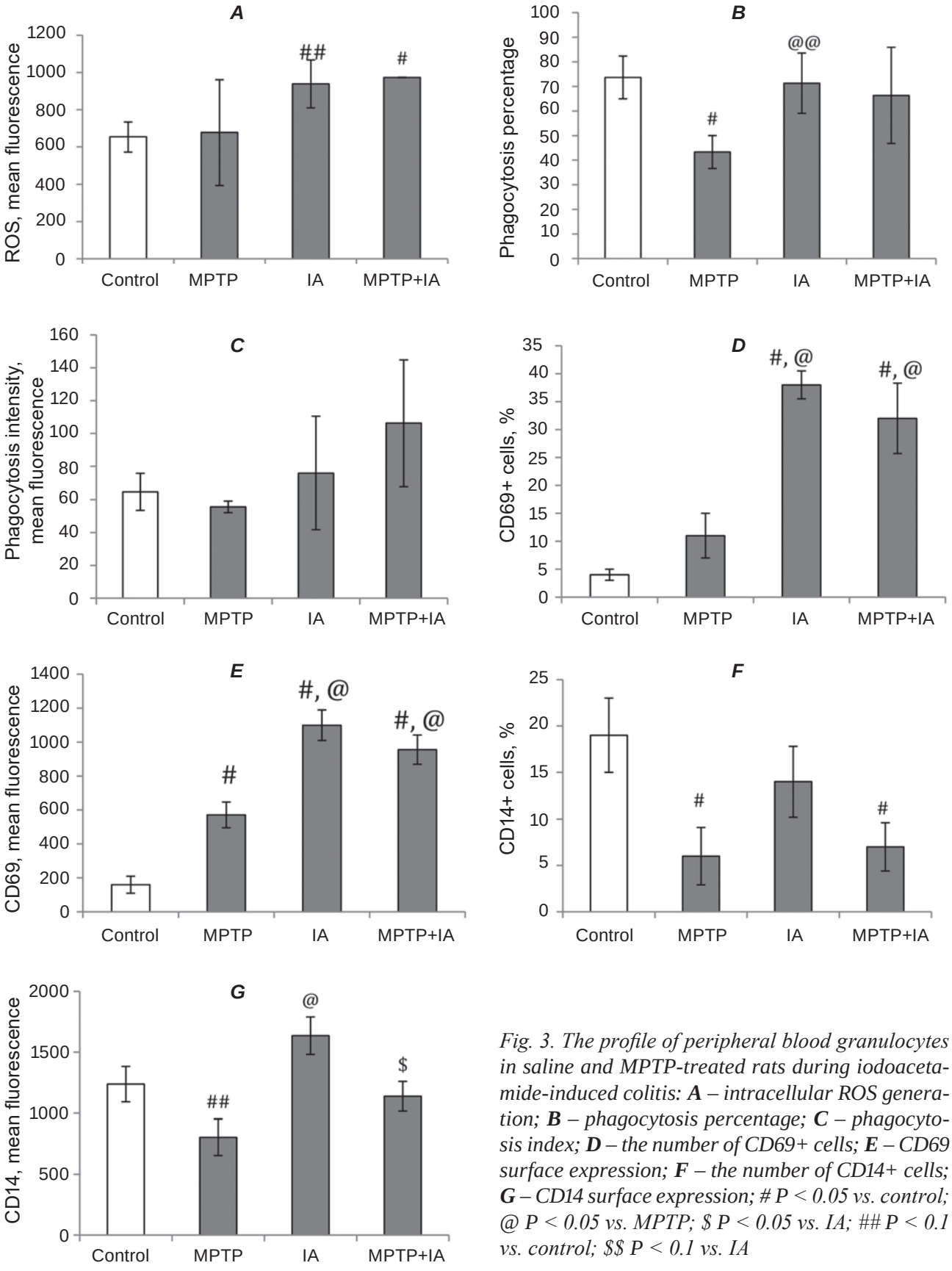

Fig. 3. The profile of peripheral blood granulocytes in saline and MPTP-treated rats during iodoacetamide-induced colitis: ***A*** *– intracellular ROS generation;* ***B*** *– phagocytosis percentage;* ***C*** *– phagocytosis index;* ***D*** *– the number of CD69+ cells;* ***E*** *– CD69 surface expression;* ***F*** *– the number of CD14+ cells;* ***G*** *– CD14 surface expression; # P < 0.05 vs. control; @ P < 0.05 vs. MPTP; $ P < 0.05 vs. IA; ## P < 0.1 vs. control; $$ P < 0.1 vs. IA*

CD69, an early activation marker antigen on T and B cells, is also expressed on activated macrophages and neutrophils. It was found that CD69-deficient mice developed less bleomycin-induced lung injury and inflammation vs. wild type mice [36]. In our study, the number of CD69 positive granulocytes (Fig. 3, *D*) and intensity of CD69 surface expression (Fig. 3, *E*) was similar in MPTP and saline-treated rats with IA-induced colitis, while were significantly higher vs. control animals. The intensity of CD69 surface expression was also increased in MPTP-treated rats without colitis (Fig. 3, *E*).

The membrane-expressed CD14 is the most important endotoxin coreceptor on phagocytic cells. In our study, destruction of peripheral dopaminergic system in MPTP-treated rats was associated with significant decrease of CD14 positive granulocytes number and intensity of CD14 surface expression vs. control animals (Fig. 3, *F, G*). Furthermore, these parameters were also significantly lower in MPTP- vs. saline-treated rats with IA-induced colitis (Fig. 3, *F, G*).

Monocytes and macrophages, together with dendritic cells, represent the mononuclear phagocyte system, which plays a key role in maintaining tissue integrity. Mononuclear phagocytes are also critical in tissue restoration after injury, as well as in the initiation and resolution of innate and adaptive immune responses [30]. DA and dopaminergic agents can affect several functions of monocytes; for example, DA is able to decrease LPS-induced proliferation of human monocytes [37]. Recently, it was shown that in LPS-stimulated bone marrow-derived macrophages, the inflammatory process is mitigated by the action of DA on D1-dopamine receptor, through the inhibition of the NLRP3 inflammasome, a cytosolic protein complex that induces inflammation in response to bacterial pathogens. Moreover, DA, acting on DR D1, can prevent systemic and neuroinflammation also *in vivo* [38].

We found that destruction of peripheral dopaminergic system in MPTP-treated rats was associated with strong decrease of ROS production in circulating monocytes (Fig. 4, *A*), while the phagocytosis activity (Fig. 4, *C*) and the number of monocytes in phagocytosis (Fig. 4, *B*) was not changed in comparison to control animals. Furthermore, ROS production in circulating monocytes was also lower in MPTP- vs. saline-treated rats with IA-induced colitis, but this parameter didn't reach statistical significance (Fig. 4, *A-C*). These data pointed out the anti-inflammatory profile of monocytes that might explain less profound colitis-associated colonic lesions in MPTP- vs. saline-treated rats.

The number of CD69 positive monocytes in saline-treated rats with IA-induced colitis didn't differ from control group (Fig. 4, *D*), wherein intensity of CD69 surface expression was 3-fold increased. While, the number of CD69 positive monocytes and intensity of CD69 surface expression were 1.8-fold and 6-fold increased, respectively, in MPTP-treated rats during colitis vs. control, MPTP-treated rats without colitis and saline-treated rats with IA-induced colitis (Fig. 4, *D, E*). Worth to mention that MPTP-treated rats without colitis had also 4-fold increased intensity of CD69 surface expression on monocytes vs. control rats (Fig. 4, *E*). Various studies on experimental colitis in CD69-deficient mice showed the development of severe colitis with increased transcript levels of pro-inflammatory cytokines [38]. Overexpression of CD69 induced the production of tolerogenic cytokines and immune-suppressive cells, which could attenuate intestinal inflammation. In our study CD69 was significantly higher expressed in MPTP-treated rats with experimental UC, which could indicate the involvement of CD69+ cells in limitation of intestinal inflammation confirming to be an important negative regulator of immune responses in gut.

At the same time the number of CD14 positive cells was significantly decreased in MPTP and IA rats and did not differ in MPTP-treated rats with experimental UC (Fig. 4, *F*). Interestingly, the CD14 surface expression was 2-fold increased in MPTP-treated rats without colitis, but significantly decreased in both saline- and MPTP-treated rats with colitis (Fig. 4, *G*). Taking into account that CD14 is the monocyte-specific marker and the number of circulating monocytes might dependent on the rate of their translocation to inflamed tissue; observed changes might indicate the increased rate of monocyte tissue translocation.

We have shown for the first time that the destruction of peripheral dopaminergic neurons leads to the improvement of morphological signs of experimental colitis in rats. One of the possible mechanisms of the observed effect might be through regulatory effect of dopaminergic system on monocytes phenotype and their respiratory burst activity.

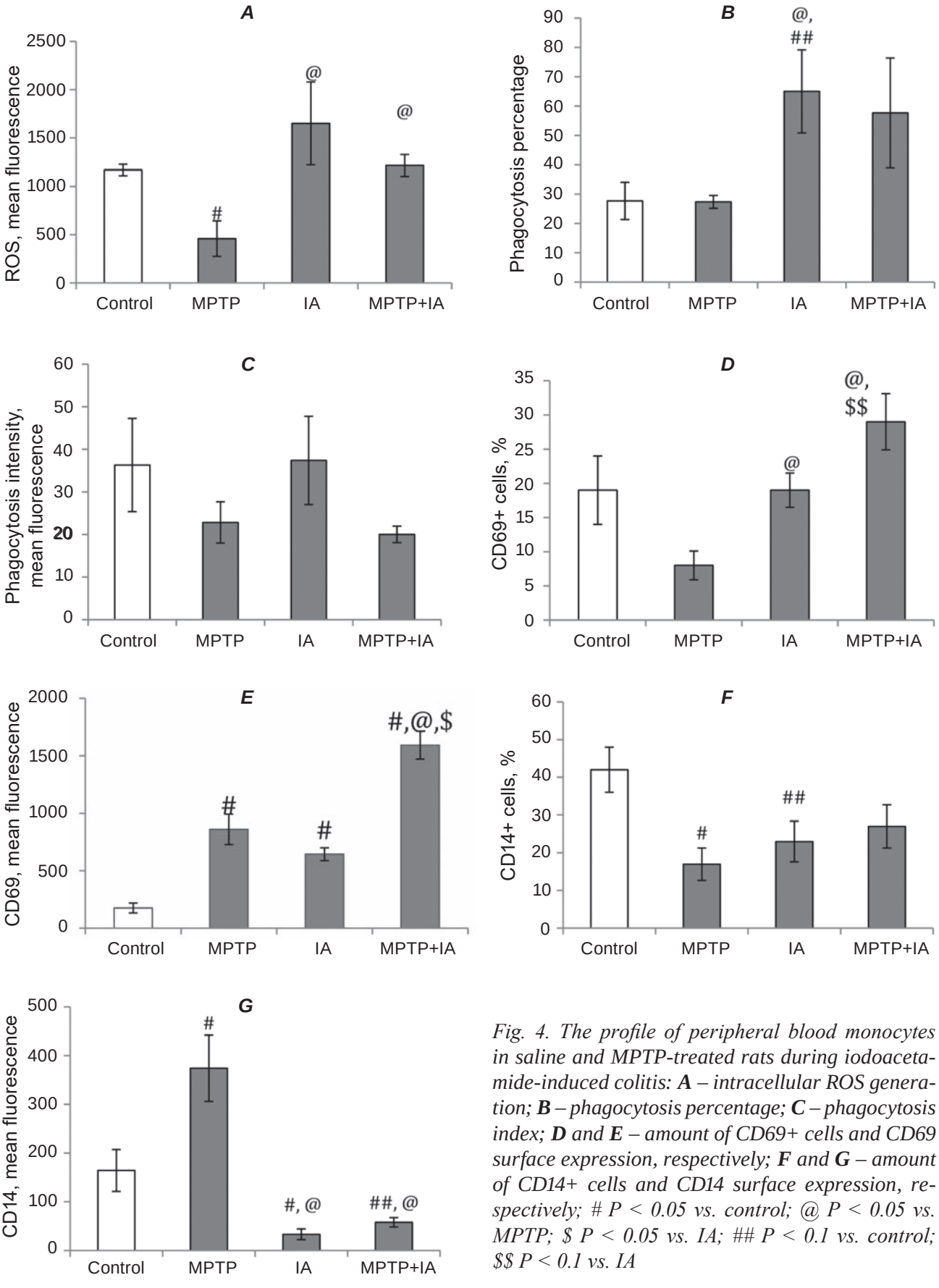

Fig. 4. The profile of peripheral blood monocytes in saline and MPTP-treated rats during iodoacetamide-induced colitis: ***A*** *– intracellular ROS generation;* ***B*** *– phagocytosis percentage;* ***C*** *– phagocytosis index;* ***D*** *and* ***E*** *– amount of CD69+ cells and CD69 surface expression, respectively;* ***F*** *and* ***G*** *– amount of CD14+ cells and CD14 surface expression, respectively; # P < 0.05 vs. control; @ P < 0.05 vs. MPTP; $ P < 0.05 vs. IA; ## P < 0.1 vs. control; $$ P < 0.1 vs. IA*

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РОЛЬ ПЕРИФЕРИЧНОЇ ДОФАМІНЕРГІЧНОЇ СИСТЕМИ В ПАТОГЕНЕЗІ ЕКСПЕРИМЕНТАЛЬНОГО КОЛІТУ В ЩУРІВ

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Дофамін (ДА) продукується і вивільнюється імунними клітинами. На сьогоднішній день відомо, що ДА є головним медіатором між нервовою та імунною системами. Метою цього дослідження була перевірка гіпотези про те, що периферична дофамінергічна система відіграє негативну роль в патогенезі виразкового коліту через вплив на активність периферичних фагоцитів крові. Дослідження проводили на щурах-самцях Вістар (170–200 г). Периферична дофамінергічна система була зруйнована ін'єкцією 1-метил-4-феніл-1,2,3,6-тетрагідропіридину (MPTP; 4×20 мг/кг, п/ш кожні 2 год). Коліт у щурів зумовлювали введенням 0,1 мл 6%-го йодоацетаміду. На 18-й день експерименту тварин піддавали аутопсії. Показано, що щури, яким вводили MPTP, мали знижені рівні тирозингідроксилази, ензиму, який лімітує синтез дофаміну в товстій кишці, але не в мозку. Число та активність гранулоцитів периферичної крові (переважно нейтрофілів) та кишкових не відрізнялися в щурів, яким вводили фізіологічний розчин та в щурів, яким вводили MPTP. За введення MPTP у щурів були знижені рівні продукції АФК моноцитами, в 1,8 раза збільшена кількість CD69 (ранній маркер активації) позитивних моноцитів та у 6 разів збільшена інтенсивність поверхневої експресії CD69 у щурів, яким вводили MPTP порівняно з контролем з експериментальним колітом. Поверхнева експресія CD14 (корецептор ендотоксину фагоцитів) була підвищеною у 2 рази в щурів, яким вводили MPTP, але вірогідно зниженою в щурів із колітом, яким вводили MPTP, та контрольних щурів із колітом. Нами було вперше показано, що руйнування периферичних дофамінергічних нейронів призвело до покращення морфологічних ознак експериментального коліту в щурів, що можна пояснити регуляторним ефектом дофамінергічної системи на фенотип моноцитів та їхньої продукції АФК.

Ключові слова: дофамінергічна система, виразковий коліт у щурів, гранулоцити, моноцити, CD69, CD14.

РОЛЬ ПЕРИФЕРИЧЕСКОЙ ДОФАМИНЭРГИЧЕСКОЙ СИСТЕМЫ В ПАТОГЕНЕЗЕ ЭКСПЕРИМЕНТАЛЬНОГО КОЛИТА У КРЫС

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Дофамин (ДА) продуцируется и высвобождается иммунными клетками. На сегодняшний день известно, что ДА является основным медиатором между нервной и иммунной системами. Целью данного исследования была проверка гипотезы о том, что периферическая дофаминэргическая система играет негативную роль в патогенезе язвенного колита посредством влияния на активность фагоцитов периферической крови. Исследования проводили на крысах-самцах Вистар (170–200 г). Периферическая дофаминэргическая система была разрушена инъекцией 1-метил-4-фенил-1,2,3,6-тетрагидропиридина (MPTP; 4×20 мг/кг, п/к каждые 2 часа). Колит у крыс вызывали введением 6%-го йодоацетамида. На 18-й день эксперимента крыс умертвили. Показано, что крысы, которым вводили MPTP, имели пониженные уровни тирозингидроксилазы, лимитирующего энзима в синтезе дофамина в толстом кишечнике, но не в мозгу. Число и активность гранулоцитов периферической крови (в основном нейтрофилов) и кишечных не отличались у крыс, которым вводили физио-

логический раствор и у крыс, которым вводили MPTP. При введении MPTP у крыс были снижены уровни продукции АФК моноцитами, число CD69 (ранний маркер активации) позитивных моноцитов было увеличено в 1,8 раз и интенсивность поверхностной экспрессии CD69 была повышена в 6 раз у крыс, которым вводили MPTP по сравнению с контролем с экспериментальным колитом. Поверхностная экспрессия CD14 (корецептор эндотоксина фагоцитов) была повышена в 2 раза у крыс, которым вводили MPTP, но достоверно снижена у крыс с колитом, которым вводили MPTP, и контрольных крыс с колитом. Нами было впервые показано, что разрушение периферических дофаминэргических нейронов привело к улучшению морфологических признаков экспериментального колита у крыс, что можно объяснить регуляторным эффектом дофаминэргической системы на фенотип моноцитов и их продукцией АФК.

Ключевые слова: дофаминэргическая система, язвенный колит у крыс, гранулоциты, моноциты, CD69, CD14.

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