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Inhibitors of Poly(ADP-Ribose)Polymerase-1 as Agents Providing Correction of Brain Dysfunctions Induced by Experimental Diabetes

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The effects of 1,5-isoquinolinediol (IQD) and nicotinamide (NAm), inhibitors of poly-(ADP-ribose) polymerase-1 (PARP-1), on inflammatory processes and activation of PARP-1 under conditions of the development of experimental diabetic neuropathy, DN (a complication of streptozotocin-induced type-1 diabetes) in rats were studied. The content of IL-4 in blood serum in the case of DN was 50% higher, while that of monocyte-chemotactic protein-1 was 90% higher than those in the control. The content of gamma-interferon also increased, while the content of the granulocyte-macrophage colony-stimulating factor did not change. Against the background of activation of PARP-1 and a decrease in the content of the substrate of this enzyme nicotinamide adenine dinucleotide (NAD) in the brain, fragmentation of PARP-1 was intensified; an increase in the ratio of the contents of a 89 kDa fragment/intact enzyme molecules proved this fact. The mentioned two structurally dissimilar PARP-1 inhibitors partly or entirely normalized the above parameters under DN conditions. These results demonstrate that PARP-1 is one of the main functional targets in realization of the effects of IQD and NAm. At the same time, the spectrum of action of these inhibitors is wider. In particular, they affect the level of proinflammatory cytokines. The ability of the investigated PARP-1 inhibitors to prevent cell death in the brain by suppressing activation and fragmentation of the above-mentioned enzyme shows that other types of action of these agents at the molecular level are possible; these may be the maintenance of the genome integrity in the brain structures under DN conditions and preventing the development of inflammatory processes. Thus, the examined inhibitors can be used in the future in the treatment of brain dysfunctions that are complications of type-1 *diabetes mellitus*.

Keywords: type-1 *diabetes mellitus*, diabetic neuropathy (DN), inhibitors of poly-(ADP-ribose) polymerase-1 (PARP-1), 1,5-isoquinolinediol, nicotinamide, cytokines, activation of PARP-1.

INTRODUCTION

Diabetes mellitus (DM) is one of the most serious endocrine diseases. Among its complications, diabetic neuropathy (DN) is the most widespread and aggravating. The development of this pathology leads to significant disability or even death of the patients [1], and its treatment is rather complicated and costly. At present, the prevalence of DN among patients suffering from DM of types 1 and 2 is 65–70% [2]. It is believed that the most immediate reasons for the development and progression of DN are long-lasting hyperglycemia and deviation of a number of metabolic processes associated with these pathological shifts. In particular, these are intensification of oxidative-nitrosative stress, advanced glycation, accumulation of its end products, essential alterations of carbohydrate and lipid metabolism, etc. [3].

The main and most frequent complication of diabetes is peripheral DN, but the development of DM also causes, as a rule, changes in the CNS, and these events are accompanied by a number of brain dysfunctions. Neurophysiological disorders associated with DN are manifested on the physiological, metabolic, structural, and molecular levels, and these pathological shifts

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are long-lasting. This situation inevitably leads to functional and structural changes in the brain, which are responsible for memory formation, mental abilities, and emotional behavior. It is obvious that such events lead to significant negative social consequences.

At present, a relatively wide range of pharmacological agents for the symptomatic treatment of peripheral DN and encephalopathies has been proposed [4]. A number of new drugs, including acetyl-L-carnitine, alpha-lipoic acid, protein kinase C inhibitors (e.g., ruboxistaurin), aldose reductase inhibitors (fidarestat), agents affecting end products of intensified glycation (aminoguanidine), those influencing the hexosamine pathway (benfotiamine), angiotensin-converting enzyme inhibitors (trandolapril), and also inhibitors of poly(ADP-ribose)polymerase, PARP-1 (nicotinamide, NAm), are being examined and used in clinical testing. Most of these drugs can act simultaneously via a few mechanisms. In particular, NAm is characterized by a rather broad spectrum of actions; this agent affects neurotransmitter systems, in particular the serotonergic system, and also the functioning of the sodium-potassium pump. We observed the respective effects in the rat brain under conditions of development of type-1 DM [5]. Despite the demonstrated cytoprotective effect of NAm (prevention of the development and suppression of DM manifestations due to restoration of the functions of pancreatic beta cells), the effectiveness of NAm as an inhibitor of PARP-1, as well as the effectiveness of other enzyme inhibitors observed under DN conditions, have not been fully elucidated [6]. It is clear that a choice of the most effective drug for the treatment of a specific DN type (encephalopathy, autonomic neuropathy, or peripheral neuropathy) is impossible without identification of all possible mechanisms of action of PARP-1 inhibitors. This has stimulated the search for some novel effective targeted drugs. Moreover, clarification of the effect of PARP-1 inhibitors on the development of DN is also important for more profound understanding of not only the mechanisms of action of these agents, but also of specific mechanisms underlying DN development. The latter mechanisms remain insufficiently explored.

As is known, chronic hyperglycemia is accompanied by significant physiological, biochemical, and histological changes in patients suffering from DM. Such changes inevitably lead to the development of endothelial dysfunctions. This, in turn, causes a decrease in the intensity of blood flow and the development of intraneuronal and intracellular hypoxia. The state of generalized hypoxia enhances the development of pathophysiological and morphological changes in brain cells under DN conditions [7]. In such a situation, dramatic changes develop in the endothelium of blood vessels that provide blood supply to nerves. In this case, other important molecular mechanisms begin to be involved; in particular, these are stress of the endoplasmic reticulum, intensified phosphorylation of mitogen-activated protein kinases (MAPKs) [8], activation of PARP-1, and activation of a few other enzymes [5]. Under these conditions, inflammatory processes can also be initiated; these are pathological events that provoke tissues to react quickly to the respective changes. This leads to the accompanying involvement of different cell types, in which regulation of expression of certain genes may be disordered because of the action of the corresponding negative factors.

Considering all the above mentioned, we examined metabolic pathways mediating the actions of two PARP-1 inhibitors, NAm and 1,5-isoquinolinediol (IQD), under conditions of the development of cerebral dysfunctions induced by experimental type-1 DM.

METHODS

Induction of experimental type-1 DM and the scheme of administration of PARP-1 inhibitors. Experimental type-1 DM in male Wistar rats, weighing 125–150 g and kept under standard vivarium conditions with free access to food and water, was induced by single intraperitoneal (i.p.) injection of 70 mg/kg body mass streptozotocin (STZ; Sigma, USA); STZ was diluted in citrate buffer (0.1 M, pH 4.5) [9]. Control rats were injected only with the buffer in a similar volume. Rats of the STZ-treated group, after 10 weeks of the development of diabetes, were everyday i.p. injected during two weeks with 3 mg/kg IQD or 100 mg/kg NAm (both from Sigma, USA). Blood samples were collected in the morning after 12-h-long starvation from the retrobulbar venous sinus of the eye; the respective manipulations were performed under light ether narcosis. Glucose levels in the blood were measured using a Precision Xtra Plus glucometer (MediSense UK Ltd., Great Britain). Only rats with clearly

expressed DM (glucose level in the blood above 17 mM) were used in the study.

Measurements of the cytokine contents in the blood serum. The respective indices were measured in the studied rats using a multiplex set, Rat Cytokines 6plex FlowCytomix Multiplex (eBioscience, USA) on a flow cytofluorometer, COULTER EPICS XL (Beckman Coulter, USA) equipped with an argon laser (λ_{excit} = 488 nm). The kit used includes spherical particles of different diameters, containing antibodies against granulocyte-macrophage colony-stimulating factor (GM-CSF), gamma-interferon (IFN-γ), interleukin-1alpha (IL-1α), interleukin-4 (IL-4), monocyte chemotactic protein-1 (MCP-1), and tumor necrosis factor alpha (TNF-α). The results were processed using FCS Express V3 (De Novo Software, USA).

Measurement of the NAD content in rat cerebral tissues. The NAD levels in rat brain tissues were determined in deproteinated acidic extracts from brain tissues using the technique based on reduction of NAD mediated with alcohol dehydrogenase. The coefficient of molar extinction (6.22 cm^2/μmol at the 340 nm wavelength) was taken into account [10].

Measurement of the protein content in the samples under study. This index was estimated according to absorption within the UV region of the spectrum using an SF-2000-02 spectrophotometer (Spectrum, Russia) according to the method described earlier [11], and also by the Bradford technique [12].

Estimation of the activity of PARP-1 in the cerebral structures of rats. The activity of PARP-1 in the nuclei of brain cells was evaluated by the amount of a radio-labeled ADP-ribose fragment, [U-^{14}C] NAD (Amersham, Great Britain), included in common nuclear proteins of the rat brain cells, according to the technique described by Tanihava et al. [13]; Whatman GF/C filters were used. Amounts of radioactivity bound during 1 min by 1 mg of nuclear proteins were measured using an SL-30 liquid scintillation counter (Intertechnique, France).

Fractioning and separation of cerebral nuclear proteins. A 0.5% solution of NP40-PBS containing a mixture of protease inhibitors and phosphatase (Thermo Scientific, USA) was added to 100 mg of brain tissue dispersed in liquid nitrogen; this caused lysis of the cell membranes. The homogenate was stirred at +4°C for 20 min [14]. The nuclei were sedimented by centrifugation (20 min, 14,000 g). A lysing buffer, RIPA, of the composition: Tris-HCl, 20 mM (pH 7.6); Triton-X100, 1%; NaCl, 150 mM; NaF, 50 mM, and sodium dodecyl sulfate (SDS), 0.2%, was added to the nuclear precipitate. The nuclear membranes were destroyed by ultrasound using a lab ionizer, Lab Sonic M (Sartorius, Germany). The procedure (20 sec) was performed twice. A 5×Laemmli buffer was added to the interphase (nuclear fraction of proteins), and the latter was warmed up in a water bath (100°C) for 5 min. Prior to use, the samples were stored at –80°C.

Electrophoresis of proteins in polyacrylamide gel (PAAG). Electrophoresis of proteins was performed during 3–4 h in PAAG (8–10%) in the presence of 0.1% SDS, using a Mini-PROTEAN II chamber for electrophoresis (BIO-RAD, Sweden) [15] for subsequent immunoblotting analysis. The molecular mass of proteins in the electrophoregrams was determined using standards of proteins obtained from Thermo Scientific (USA) and Fermentas (Lithuania).

Immunoblotting of proteins. Blotting of proteins from PAAG on a nitrocellulose membrane (GE Healthcare, Great Britain) was performed on a Mini Trans-Blot Cell device (BIO-RAD, Sweden) at a voltage of 100 V during 90 min. At the end of the process, the membrane was stained during 5–10 min with a 1% solution of Ponceau S dye prepared on a 3% solution of trichloroacetic acid. Then free binding sites on the membrane were blocked during 60 min by a 5% solution of skim milk powder (APEX Research, USA) in PBS buffer with the addition of 0.1% Tween-20 (PBSt). Subsequently, the membrane was incubated overnight with prim-ary antibodies in the buffer for blocking at +4°C; this was followed by washing off with PBSt (three times for 5 min). As secondary antibodies, anti-mouse or anti-rabbit IgGs, conjugated with horseradish peroxidase, in dilutions 1:10000 and 1:1000 in the blocking buffer, respectively, were used. Incubation with secondary antibodies was carried out for 60 min at room temperature, and then the membrane was washed off with PBSt three times for 5 min. The following antibodies were used in the study: anti-Poly (ADP-ribose) (Trevigen, USA), anti-insulin (Millipore, USA), anti-β-actin-peroxidase (Sigma, USA), anti-lamin B1 (Abcam, Great Britain), anti-PARP (Cell Signaling Technology, USA), anti-mouse IgG (Sigma, USA), and anti-rabbit IgG (Sigma, USA). Immunoreactive zones were detected

by measuring the intensity of chemiluminescence [16]. Densitometric analysis was performed using TotalLab TL120 software (Nonlinear Inc., USA). The protein contents are shown below in arbitrary units (a.u.).

Evaluation of the expression of PARP-1 mRNA in rat brain tissues using the polymerase chain reaction (PCR). When using PCR with reverse transcriptase (RT), total samplings of RNA were obtained from 100 mg of chopped brain tissue using a set of reagents, Trizol (Isogene, Russia), according to the manufacturer's protocol. Complementary DNA (cDNA) was obtained according to the published method [17] using the QuaniTect Reverse Transcription kit (QIAGEN, Germany). The resulting cDNA was used for quantitative PCR according to the respective method [18]. For amplification of PARP-1 mRNA, direct primers (5'-AAGGTCAAGAAGACCGCAGA-3 ') and reverse primers (5'-AGAGGAGGCTAAAG CCCTTG-3') were used. The nucleotide sequences in these primers correspond to sequences 312–331 and 624–605 in rat cDNA PARP-1 (GenBank No. NM_013063). The intensity of expression of β-actin mRNA served as an additional control of the amount of RNA taken for analysis. For amplification of β-actin mRNA, direct primers (5'-CGTACCACTGGCATCGTGAT-3 ') and reverse primers (5'-GTGTTGGCGTACAGGTCTTT-3') were used. The PCR was performed on a Stratagene Mich 3000P cycler (USA) using SYBRGreen Mich (AB gene, Great Britain).

Numerical results were analyzed using a special computer program, Differential Expression Calculator. Statistical analysis was performed using MS Excel. Mean arithmetic values of the numerical data were obtained in three to five independent experiments; s.e.m. values were also calculated. The intergroup comparison was performed using the Student's *t*-test. The respective differences were considered significant at $P < 0.05$.

RESULTS

Estimations of the glucose levels in the blood of animals of the studied groups allowed us to determine the validity of the experimental DM model used. The body mass of the rats and the level of glucose in their blood at the beginning of the experiments were practically similar to each other in all the groups studied. After 12 weeks of the development of STZ-induced type-1 DM, the mean body mass of diabetic rats was 24.3% smaller, on average, and blood glucose levels corresponded to about 370%, as compared to the corresponding values in control animals (see Table 1). In other words, single STZ injections induced the development of strong uncompensated hyperglycemia.

Chronic administration of PARP-1 inhibitors (IQD or NAm) to control rats evoked no considerable shifts in the body mass of animals of both groups and in the glucose concentration in their blood. Administration of these inhibitors also did not affect the body mass in animals with DM (under the action of the investigated PARP-1 inhibitors, this parameter did not increase, as compared with that in diabetic rats that were not treated with these agents). The use of these inhibitors also practically did not reduce the level of glucose in the blood of such animals.

As is known, hyperglycemia caused by type-1 DM may result not only from the development of pancreatic β-cell dysfunction induced by the action of various endo- and exogenous factors,

Table 1. Mean Body Mass of Rats and Glucose Level in their Blood (M ± s. d.; n = 5)

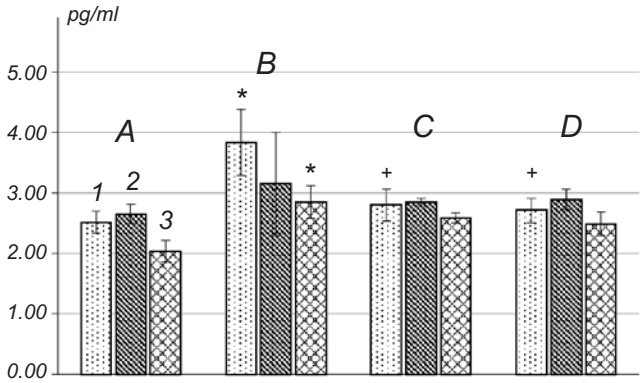
Groups	Body mass, g		Glucose level, mM	
	initial	at the end of the 12th week	initial	at the end of 12th week
Control	110 ± 12	353 ± 27	5.1 ± 0.5	5.3 ± 1.5
Rats with *diabetes mellitus* (DM)	112 ± 9	288 ± 23*	5.1 ± 0.5	19.4 ± 2.3*
Rats with DM treated with 1,5-isoquinolinediol (IQD)	109 ± 10	223 ± 10+	5.1 ± 0.5	18.8 ± 1.4
Rats with DM treated with nicotinamide (NAm)	110 ± 10	286 ± 15	5.1 ± 0.5	18.0 ± 1.7

Footnotes: *$P < 0.05$ in comparisons with the control group, +$P < 0.05$ in comparisons with rats of the DM group.

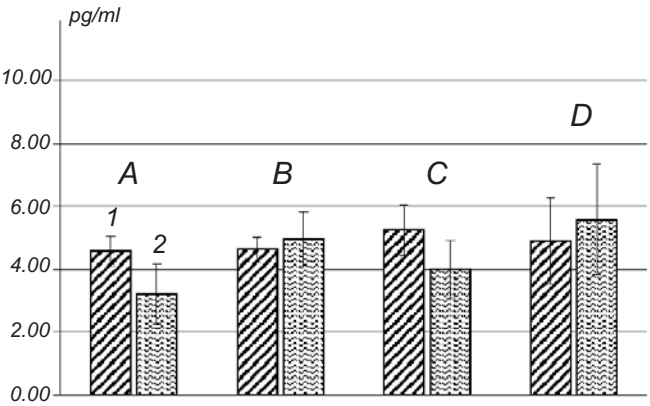
which ultimately leads to the loss of the mentioned cells. This effect (hyperglycemia) can also occur due to increase in the content of proinflammatory cytokines in the circulation [19]. Despite the fact that cytokines in healthy subjects are present only in trace amounts, their effects can be quite significant because their effects are realized through specific high-affinity receptors localized on the outer surface of the cytoplasmic cell membranes. Formation and secretion of cytokines are precisely regulated, and this provides adequate intercellular and intersystem interactions in the organism. This allows the latter to maintain normal levels of cell survival in different tissues, stimulation or suppression of cell growth, cell differentiation, adequate cellular functional activity, and normal intensity of apoptosis. Cytokine-related control also provides coordination of the functioning of the immune, endocrine, and nervous systems, both under physiological conditions and upon the action of various external and internal factors [20, 21]. The spectra of manifestations of biological activity of the cytokines can, in many cases, overlap, since different cytokine-receptor complexes can activate the same protein carriers in the signaling pathway. The role of anti-inflammatory cytokines in the pathogenesis of type-1 DM increases significantly within the late stages of this pathology, i.e., in the presence of DN. This aspect, as well as the possible ability of PARP-1 inhibitors to affect these cellular agents, is still not fully clarified.

The content of IL-4 in the blood serum of experimental rats in the case of DN was about 50% higher than in the control; the content of IFN-γ also was increased. The administration of IQD provided a decrease only in the content of IL-4; NAm did not affect the studied parameters. During the development of DN, we also did not observe significant changes in the concentration of GM-CSF (Fig. 1). The latter is a growth factor that stimulates the functional activity of neutrophils, eosinophils, and monocytes; it can be produced in tissues of various types.

Since TNF-α, the factor produced by monocytes and macrophages, is involved in the pathogenesis of many diseases, in particular of Alzheimer's disease (in which a relationship between the levels of TNF-α and β-amyloid protein was found [22, 23]), it was expedient to measure the level of this factor in the blood serum of animals with DN. It turned out, however, that the TNF-α content in such rats did not change, as compared with the control. At the same time, the content of IL-1α (an extracellular peptide) showed a clear increase (Fig. 2). Type-1 interleukins (IL-1) are biologically active proteins produced by macrophages and T-lymphocytes, which are involved in the development of inflammatory reactions and the immune response of the organism. Under the action of a wide range of cytokines, in particular IL-6, TNF-α, IL-1β, and a monocyte chemotactic protein-1 (MCP-1) is produced in the organism. Due to its specificity with respect to target cells, MCP-1 plays an important role in the development of various diseases (Alzheimer's disease, atherosclerosis, viral infections, etc.). As we have found, the content of MCP-1 in blood serum of rats with DM-induced DN increased and reached a 53.57 ± 6.79 pg/ml level vs. 28.8 ± 8.16 pg/ml



F i g. 1. Contents of interleukin-4 (IL-4, 1), granulocyte-macrophage colony-stimulating factor (GM-CSF, 2), and gamma-interferon (INF-γ, *3*) in the blood serum, pg/ml, of rats of the control group (A), animals with experimental type-1 *diabetes mellitus* (B), and diabetic rats treated with 1,5-isoquinolinediol (C) and nicotinamide (D). $^{*}P < 0.05$ in comparisons with the control group; $^{+}P < 0.05$ in comparisons with diabetic rats.



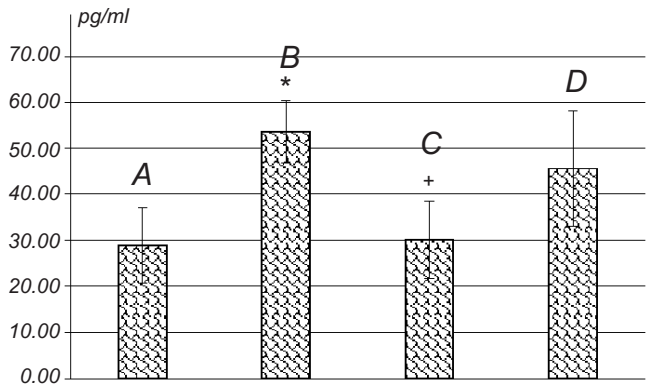
F i g. 2. Contents of tumor necrosis factor-alpha (TNF-α, 1) and interleukin-1α (IL-1α, 2) in the blood serum of rats. Designations are similar to those in Fig. 1.

in the control, i.e., an almost twofold rise was observed (Fig. 3). The chronic administration of IQD led to a clear trend toward normalization of this parameter. At the same time, the effect of NAm was insignificant from this aspect.

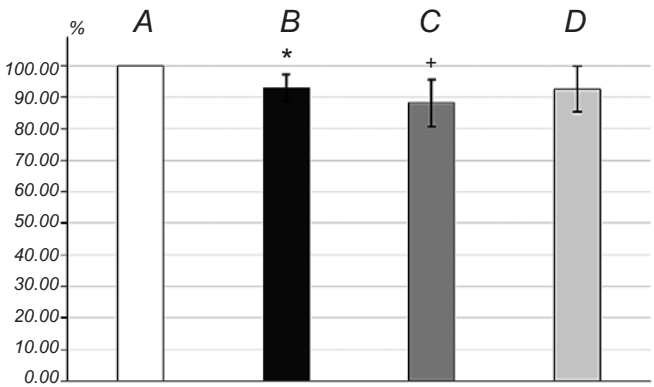
Poly-ADP-ribosylation in brain tissues under conditions of development of DN. It cannot be ruled out that endogenic ADP-ribosylation of nuclear proteins, i.e., one of the key mechanisms of DN development, is intensified against the background of inflammation processes related to this pathology. In our previous studies, it has been shown that type-1 DM in rats is associated with intensification of oxidative stress in the brain and activation of poly-ADP-ribosylation of nuclear proteins (a response to DNA damage) [24]. However, the question on the relation between the processes of poly-ADP-ribosylation of proteins and cerebral dysfunctions in the case of the DN development remains open. This is why we tried to estimate not only the level of poly-ADP-ribosylation of proteins in the brain under DN conditions, but also the modulation of this process by specific PARP-1 inhibitors. We believe that the use of these agents can be a new pharmacological approach in the treatment of DM complications.

As was found, the development of DN, at almost unchanged expression of mRNA of the *PARP-1* gene (Fig. 4), led to intensification of apoptotic processes in the rat brain. An enhanced intensity of proteolytic splitting of PARP-1 shown by an increase in the ratio between the amounts of the 89 kDa PARP fragment and of nonsplit molecules of the enzyme confirms this statement (Fig. 5). The inhibitors used did not affect significantly the level of expression of mRNA of the *PARP-1* gene in the brain of rats with DM (Fig. 4). At the same time, prolonged administration of these inhibitors was accompanied by suppression of the enzyme activity and, accordingly, by inhibition of proteolytic splitting of PARP-1. A noticeable decrease in the above-mentioned ratio of the contents of the 89 kDa fragment vs. intact enzyme molecules confirms this conclusion. (Fig. 5).

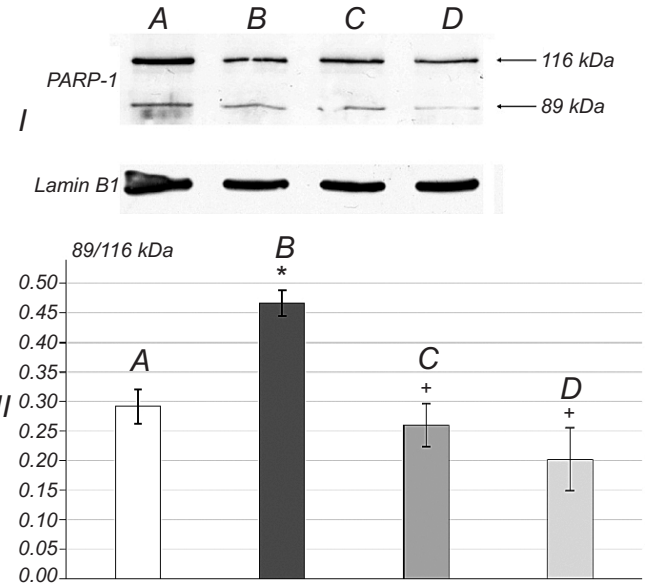
The elevated content of the 89 kDa fragment of PARP-1 in the brain of rats with DM (Fig. 6) may



F i g. 3. Content of monocyte chemotactic protein-1 (MCP-1, pg/ml) in the blood serum of rats. Designations are similar to those in Fig. 1.

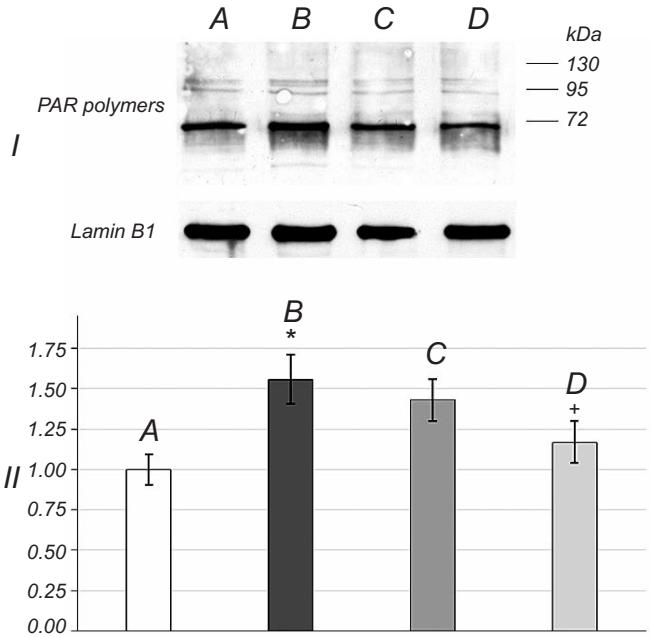


F i g. 4. Normalized intensity of expression of mRNA of the *PARP-1* gene in the brain of rats with type-1 *diabetes mellitus*, % (control values are taken as 100%). Designations are similar to those in Fig. 1.

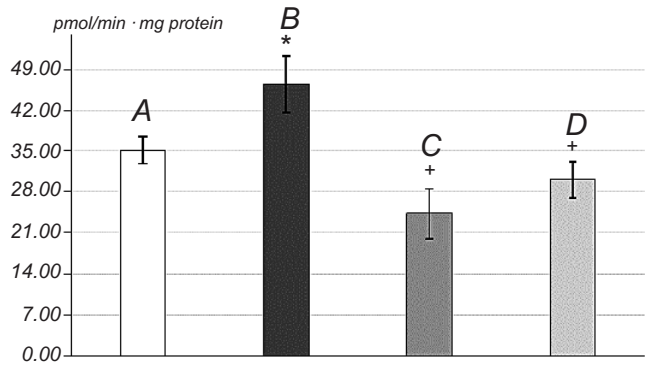


F i g. 5. Western blotting of the 89 kDa fragment and whole molecules of PARP-1 (116 kDa) in the nuclear extract from rat brain tissues. I) Blottogram, II) results of densitometry, $M \pm s.d.$ (n = 4–5). Other designations are similar to those in Fig. 1.

be a result of intensified activation of the enzyme and increase in the content of poly-ADP-ribosylated proteins (PARs). The latter may appear at the stage of reparation of DNA damages; these proteins can be involved in the regulation of gene transcription. The process of poly-ADP-ribosylation is involved in the remodeling of the chromatin structure [25]; it may also result from a protective reaction of the organism to excessive exhaustion of the energy resources of the brain cells. Indeed, according to our data, these results correlate with not only the PARP-1 activity in the cerebral nuclei of rats (Fig. 7) but also with the NAD level in these structures (Fig. 8).



F i g. 6. Western blotting of poly-ADP-ribosylated proteins in the nuclear extract from rat brain tissues. Designations are similar to those in Fig. 5.



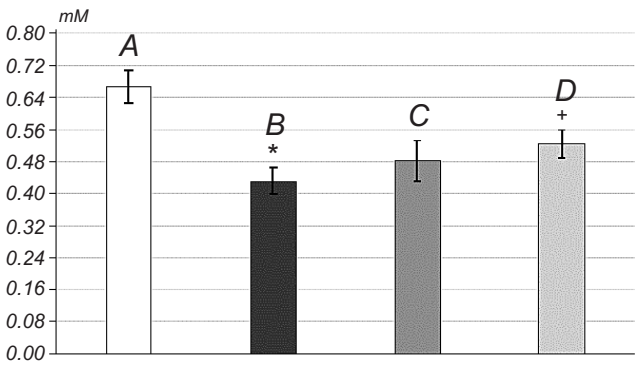
F i g. 7. Activity of PARP-1 in the nuclei of the rat brain.

Both inhibitors of PARP-1, IQD, and NAm, considerably inhibited the activity of this enzyme, but the effect of IQD was more intense (Fig. 7). With the development of DN, the content of NAD in the brain of animals with this pathology was reduced by 33.4%, on average, as compared to the control. The use of NAm resulted in an increase in the NAD content in the brain of these rats. This effect was completely expected, since administration of this inhibitor contributed to the replenishment of the pool of the mentioned dinucleotide, which is extremely necessary for physiologically normal functioning of key metabolic processes. Administration of IQD to rats with DM did not change significantly the content of this dinucleotide in the brain (Fig. 8).

DISCUSSION

Taking into account the obtained data, it becomes obvious that there are explicit functional connections between hyperglycemia, the development of inflammatory processes in the organism, and activation of PARP-1 accompanied by fragmentation of the molecule of this enzyme. Disturbances of such relationships lead to the development of DN in general and encephalopathy in particular. The use of specific PARP-1 inhibitors corrects (while only partly) the examined altered processes. This situation stimulates the search for new inhibitors of this enzyme, as well for possible modes of combining the actions of such agents and other biologically active compounds, which will contribute to more effective treatment of DN.

Against the background of the development of hyperglycemia in type-1 DM, the observed changes in the content of the studied cytokines can occur not only due to the existence of inflammatory



F i g. 8. Content of NAD^+ in the rat brain. Designations are similar to those in Fig. 1.

processes induced by diabetes of this type, but also due to the influence of other modulatory factors. Intensified synthesis of the mentioned cytokines can be induced by infectious, viruses, microorganisms (bacteria), products of their life, toxins, metabolites, some proteins of plant origin, food and medicinal allergens, and also modified cells of the organism *per se*. Moreover, the observed increase in the content of IFN-γ, IL-1α, and MCP-1 in the blood serum of rats with DM suggests that, in the case of DN, significant changes in the functional activity of immunocompetent cells of various types and the development of proinflammatory processes occur. The use of IQD resulted in a decrease in the MCP-1 level, which is likely a manifestation of more pronounced anti-inflammatory activity of the above inhibitor as compared with that of NAm. The significant increase in the content of IL-4 in blood serum (by 50%) in animals with DN can be due to the fact that IL-4 not only provides a certain anti-inflammatory effect, but also plays an important modulatory role in the chronic course of the disease. The level of this cytokine may be one of the manifestations of a compensatory response of the organism. It is known that, in the case of a significant restriction of the caloric content of consumed food, the content of IL-4 is reduced [26]. Moreover, IL-4 is an antagonist of IFN-γ, a homodimeric glycoprotein produced under inflammatory conditions by cytotoxic T-cells (CD_8), as well as by CD_4 cells of a specific subtype [27]. The content of IFN-γ also increased under conditions of our experiments. Intensification of inflammatory processes in various organs under DN conditions is one of the factors inducing intensification of angiogenesis [28]. Our observations, as well as those of other authors, indicate that the specific inhibitor PARP-1 (IQD) has the ability to prevent intensification of angiogenesis in some types of retinopathies, as well as in the case of metastases of malignant tumors [29, 30].

There are reasons to believe that poly-ADP-ribosylation of nuclear proteins in the rat brain under DN conditions is accompanied by fragmentation of the molecules of this enzyme. The activation of PARP-1 leads to a decrease in the level of its substrate, NAD. The latter, in turn, slows down glycolysis, suppresses the functioning of the electron transport chain, and reduces the formation of ATP. These events can intensify the death of brain cells. Obviously, PARP-1 plays an important regulatory role in the development of CNS dysfunctions [31]. In the case of late stages of type-1 diabetes, the imbalance between the processes of excitation and inhibition of neurons resulting from the disturbance of the process of exocytosis represents one of the main causes of the development of diabetic encephalopathy. This was evidenced by the results of our previous studies [32]. The activation of the processes of poly-ADP-ribosylation of nuclear proteins, which is accompanied by fragmentation of PARP-1 against the background of the development of inflammatory processes, confirms the above conclusion. There are reasons to believe that specific PARP-1 inhibitors examined in our study are capable of not only reducing the activity of the indicated enzyme, but also of suppressing the development of inflammatory processes. In addition to the observed effect on these processes, the inhibitors studied, as we have shown earlier, reduce the intensity of manifestations of oxidative stress in blood leukocytes in type-1 DM in rats [5]. Our data agree with the results of other authors who also demonstrated a fairly wide range of the effects of PARP-1 inhibitors [33]. Thus, with the use of NAm, which inhibits PARP-1 activity in the rat brain, the positive effect of this agent can also be mediated by inhibition of the synthesis of lipids [34]. The fact that NAm prevents PARP-1 degradation and promotes DNA repair due to direct inhibition of caspase-3 (this was shown in nerve cells) suggests that its cytoprotective action on rat brain cells can be realized via a similar way [35, 36]. Moreover, NAm administration facilitates the functioning of other regulatory NAD-dependent processes by preventing the exhaustion of the NAD pool; NAm is a precursor in NAD synthesis [37–39]. It should also be mentioned that IQD is also capable of inhibiting inducible NO synthase; thus, it is possible that this agent can prevent the formation of peroxynitrite, an important cytotoxic oxidant [40].

According to other authors, the mechanism of action of IQD on DM-induced dysfunction of the rat brain can also be realized through a positive effect of this inhibitor on other molecular events in neurons; IQD is capable of inhibiting lipid peroxidation; it improves the functioning of acetylcholinesterase and partially normalizes the levels of GABA and glutamate in the hippocampus [41].

It is also possible that receptors of voltage-dependent calcium channels are a pharmacological target for both NAm and IQD [38]. It is important that the mechanisms of PARP inhibitors work on both subcellular and cellular levels; the respective

data were obtained with the use of cell cultures [42].

The use of specific inhibitors of PARP-1 allowed us to detect their modulatory effect on both DM-induced inflammatory processes and the examined process of poly-ADP-ribosylation of nuclear proteins. In other words, the data obtained suggest that the activity of PARP-1 is increased not only at the necessity of repair of gaps in the structure of DNA molecules, which is possible under DN conditions [24, 43], but also as a result of intensification of oxidative and nitrosative stress and activation of MAP kinases [44]. At present, however, it remains practically unclear due to which mechanisms activation of PARP-1 develops. According to the existing data, PARP-1, under conditions of oxidative-nitrosative stress, interacts with glyceraldehyde-3-phosphate dehydrogenase; the latter is translocated to the nucleus under both *in vitro* and *in vivo* conditions [45]. The cited authors believe that glyceraldehyde-3-phosphate dehydrogenase is a key regulator of PARP-1 activity. Disorders in the interaction of these enzymes significantly reduce the excessive activation of PARP-1; this protects the brain from damage under conditions of stroke modeled in rats. It is possible that the same scenario is implemented under conditions of development of DN. Intensification of PARP-1 activity and formation and accumulation of the fragments of its molecules is a result of enhanced proteolytic splitting of the latter. Such a situation may lead to a unique caspase-independent form of cell death in the brain. This form is called *Parthanatos*, since translocation of apoptosis-inducing factor (AIF) from the mitochondria into the nucleus occurs under such conditions. [46].

Our results suggest that the protective effect of the investigated PARP-1 inhibitors on brain dysfunctions is realized not only due to the specific action of these inhibitors, but also due to their influence on other metabolic processes, as well as on the structure and functions of the neuronal membranes. That is why many PARP-1 inhibitors have increasingly begun to be used as anti-tumor agents applied in both individual mode and complex therapy [47, 48].

Summarizing the obtained data, we can state that, under conditions of development of DN, such a nuclear enzyme as PARP-1 is one of the main functional targets for the action of 1,5-isoquinolinediol and nicotinamide. The ability of these PARP-1 inhibitors to prevent the death of brain cells through the *Parthanatos* mechanism suggests that these agents possibly realize their action via other pathways at the molecular level. They contribute to maintaining the genome integrity in the cells of cerebral structures under DN conditions and prevent the development of inflammatory processes. Thus, these agents can find definite use in the treatment of DN.

The study was carried out in compliance with the provisions of the European Convention for the protection of animals used for experimental research and other scientific purposes (86/609 of the EEC, 1986, Strasbourg) and in accordance with the "General Ethical Principles of Animal Experiments" adopted at the 1st National Congress on bioethics (Kyiv, 2001).

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