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SCIENTIFIC ADVANCEMENT ON THE WAY TO MOLECULAR VITAMINOLOGY AT THE DEPARTMENT OF VITAMINS AND COENZYMES OF THE PALLADIN INSTITUTE OF BIOCHEMISTRY

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Modern advances in molecular vitaminology are characterized by a marked expansion in understanding the molecular mechanisms underlying the actions of vitamins and their biologically active derivatives as highly effective compounds that ensure controlled interactions between cellular regulatory systems and metabolic processes. The molecular mechanisms of the pleiotropic effects of the hormonally active form of vitamin D_3, calcitriol ($1\alpha,25(OH)_2D_3$), are realized in target tissues through vitamin D_3 receptors (VDR), which are present in virtually all cells. Our studies have focused on VDR-mediated effects, including modulation of the transcriptional activity of NF-κB, NFAT, HIF-1 and PPAR, as well as involvement of regulatory pathways such as HIF-1α/VEGF and RANK/NF-κB. We have also examined signaling through glucocorticoid and mineralocorticoid receptors, which play a key role in the antioxidant, anti-inflammatory, and anti-apoptotic effects of vitamin D_3 under normal conditions and in pathology (osteoporosis, neurodegenerative disorders associated with glucocorticoid-induced neurotoxicity and type 2 diabetes mellitus). The mechanisms of the neurotropic effects of vitamin B_3 (nicotinamide) and a derivative of nicotinic and amino butyric acid, nicotinoil-GABA (N-GABA), have also been studied. It has been demonstrated that nicotinamide (NAm) inhibits the development of diabetic neuropathy by reducing the activity and level of the PARP-1 enzyme, suppressing its fragmentation and preventing DNA damage in the brain tissue, and normalizing the nuclear levels of SIRT1 and SIRT2 proteins in neurons. One of the effective methodological approaches in our studies has been the investigation of thiamine-binding proteins in the brain and the effects of thiamine deficiency on the expression and state of neurospecific proteins. Based on our findings, we have formulated a working hypothesis regarding the molecular mechanisms of vitamin B_1 involvement in the functioning of the cholinergic component of the nervous system. This hypothesis suggests that, in addition to the pool of thiamine diphosphate (ThDP) that binds to ThDP-dependent enzymes, nerve cells contain a rapidly exchangeable pool of thiamine derivatives that are involved in acetylcholine metabolism. The research achievements of our Department demonstrate the therapeutic potential of vitamins D_3, B_3, B_1, and their biologically active derivatives in preventing the development of neurodegenerative complications under various pathological conditions and provide a scientific basis for the development of novel vitamin supplements.

K e y w o r d s: Vitamin D_3, vitamin B_3, nicotinoyl-GABA, vitamin B_1, thiamine diphosphate, glucocorticoids, transcription factors, neurospecific proteins, thiamine binding proteins, oxidative stress, inflammation, neurodegeneration, diabetes mellitus.

The current progress in molecular vitaminology is marked by the transition from the perception of vitamins as absolutely necessary components of heterotrophic organism nutrition to the understanding of their role as highly effective bioregulators for the functioning of living systems. Vitamins and their biologically active derivatives are known to be powerful naturally occurring biological molecules that maintain the structural and functional organization of cells, integrate metabolic pathways with the processes of intracellular signaling, differentiation, proliferation and apoptosis under normal and pathological conditions.

The main directions of vitaminology in Ukraine and, in particular, at the O. V. Palladin Institute of Biochemistry of the NAS of Ukraine were initiated and further developed thanks to outstanding Ukrainian vitaminologists. O. V. Palladin started research on the biochemistry of vitamins from the very beginning of the foundation in Kharkov in 1925 of the Ukrainian Biochemical Institute of the People's Commissariat of Education of the Ukrainian SSR, now the O. V. Palladin Institute of Biochemistry. The following were O. V. Palladin's pioneering publications: "On the Role of Vitamins in Nutrition" [1] and "The Chemical Nature of Vitamins" [2].

In December 1944, on the initiative of Academician O. V. Palladin, a laboratory of vitamin biochemistry was created, and in 1966 it was reorganized into the Department of the Biochemistry of Vitamins; in 1976 it was renamed the Department of Biochemistry of Coenzymes, and since 2011 it has been called the Department of Vitamins and Coenzymes Biochemistry.

The heads of the department have always been brilliant scientists who have developed original directions in classical and up-to-date vitaminology. Prof. Sergei Vinokurov (1945-1948) studied the ways of transformation of ascorbic acid (vitamin C) into dehydroascorbic acid and then into diketogulonic acid, as well as the antioxidant properties of vitamin C.

Academician Rostislav Chagovets (1948-1976) substantiated the theory of the necessity of exogenous vitamin intake due to the loss of genes of protein enzymes encoding the synthesis and biotransformation of substances of exogenous origin; developed the theory of the formation of nutrition types in the process of evolution; scientifically substantiated the harm of the use of excessive doses of vitamins in medicine. Prof. Askar Khalmuradov (1976-1985) initiated the study of non-coenzyme functions of water-soluble (coenzyme) vitamins: participation of NAD^+ in the processes of mono- and poly-ADP-rybosylation of membrane, cytosolic and nuclear proteins; postulated a hypothesis of the neurotropic function of NAD^+ in modulating the release of neurotransmitters by nerve endings; envisaged the participation of biologically active derivatives of thiamine in the regulation of acetylcholine metabolism in nerve cells. Prof. Georgy Donchenko (1986-2015) developed an understanding of the molecular mechanisms of the biological action of vitamins and coenzymes and their specific acceptor proteins in ensuring the functioning and viability of cells under normal conditions and in various pathologies; studied the involvement of tocopherol in the biosynthesis of ubiquinone and its regulatory effect on the activity of enzymes of the electron transport chain.

Currently, the research of the Department of Vitamin and Coenzyme Biochemistry under the supervision of Prof. Mykola Veliky (since 2016) is related to the study of the molecular mechanisms of the neurotropic action of biologically active forms of water-soluble vitamins B_1 (thiamine, thiamine diphosphate and thiamine triphosphate) and B_3 (nicotinamide, nicotinic acid), as well as fat-soluble vitamin D_3 and its hormonally active derivative calcitriol (1α,25-dihydroxycholecalciferol or $1\alpha,25(OH)_2D_3$).

Pleiotropic effects of vitamin D_3 in regulating cellular functions in normal and vitamin D_3-deficient states

Vitamin D_3 status, metabolism and genomic action. The results of large-scale cohort studies show a pandemic prevalence of vitamin D_3 (D_3) deficiency among the population of almost all countries in the world. Data from recent years indicate that D_3 deficiency among the world's population ranges from 24 to 49% (according to the content of $25(OH)D_3$ in the blood serum less than 50 nmol/l) [3, 4]. Epidemiological studies conducted in Ukraine at the Scientific and Medical Center for Osteoporosis also showed a high level of insufficiency (27.4%) and deficiency (19.9%) of vitamin D_3 among the examined adult population [5].

Cholecalciferol enters the body from food or is synthesized in the skin under the influence of shortwave ultraviolet radiation and is transported to tissues in a complex with vitamin D_3 binding protein (VDBP). The conversion of D_3 into its hormonally active form is carried out by two-stage hydroxylation of cholecalciferol with the participation of cytochromes P450, responsible for the production of $25(OH)D_3$ (calcidiol) and the active hormonal form $1\alpha,25(OH)_2D_3$ (calcitriol) [6]. Hydroxylated metabolites ($25(OH)D_3$, $1\alpha,25(OH)_2D_3$), biotransformation enzymes (CYP27A1, CYP2R1, CYP27B1, CYP24A1, VDBP and the vitamin D_3 receptor (VDR) are thought to be key components of the vitamin D_3 auto-/para-/endocrine system.

The hormonal form of D_3 determines the implementation of numerous biological effects in target tissues by regulating VDR-mediated gene transcription (genomic mechanism) and rapid non-genomic responses, which are carried out through the influen-

ce on the calcium-phosphate homeostasis system, ion channels, and intracellular signaling pathways [7-9].

A generalized scheme of the participation of vitamin D_3 auto-/para-/endocrine system in the regulation of cellular functions is presented in Fig. 1.

Vitamin D_3 affects bone metabolism and remodeling. Vitamin D_3 deficiency is currently considered a universal risk factor for the development of numerous multifactorial diseases. In experimental osteoporosis models (nutritional, post-immobilization, glucocorticoid-induced), we demonstrated a significant decrease in the serum level of $25(OH)D_3$, a valid marker of D_3 bioavailability in the body of animals, which indicates the development of severe D_3 deficiency in various forms of osteoporosis. One of the key reasons for the lack of circulatory $25(OH)D_3$ was significant inhibition of the activity of vitamin D_3 25-hydroxylase in hepatocytes due to the suppression of the synthesis of CYP2R1 and CYP27A1 proteins – microsomal and mitochondrial isoforms of the enzyme, respectively [10, 11]. Lowered D_3-vitamin status of animals with osteoporosis was accompanied by alterations in the expression level of VDBP, key enzymes responsible for local formation and degradation of calcitriol in tissues (CYP27B1 and CYP24A1), as well as VDR, through which autocrine and paracrine effects of $1\alpha,25(OH)_2D_3$ are realized [12, 13]. The content of these components decreased or compensatorily increased depending on the tissue and type of osteoporosis.

The goal of further studies was to establish a link between insufficient bioavailability of vita-

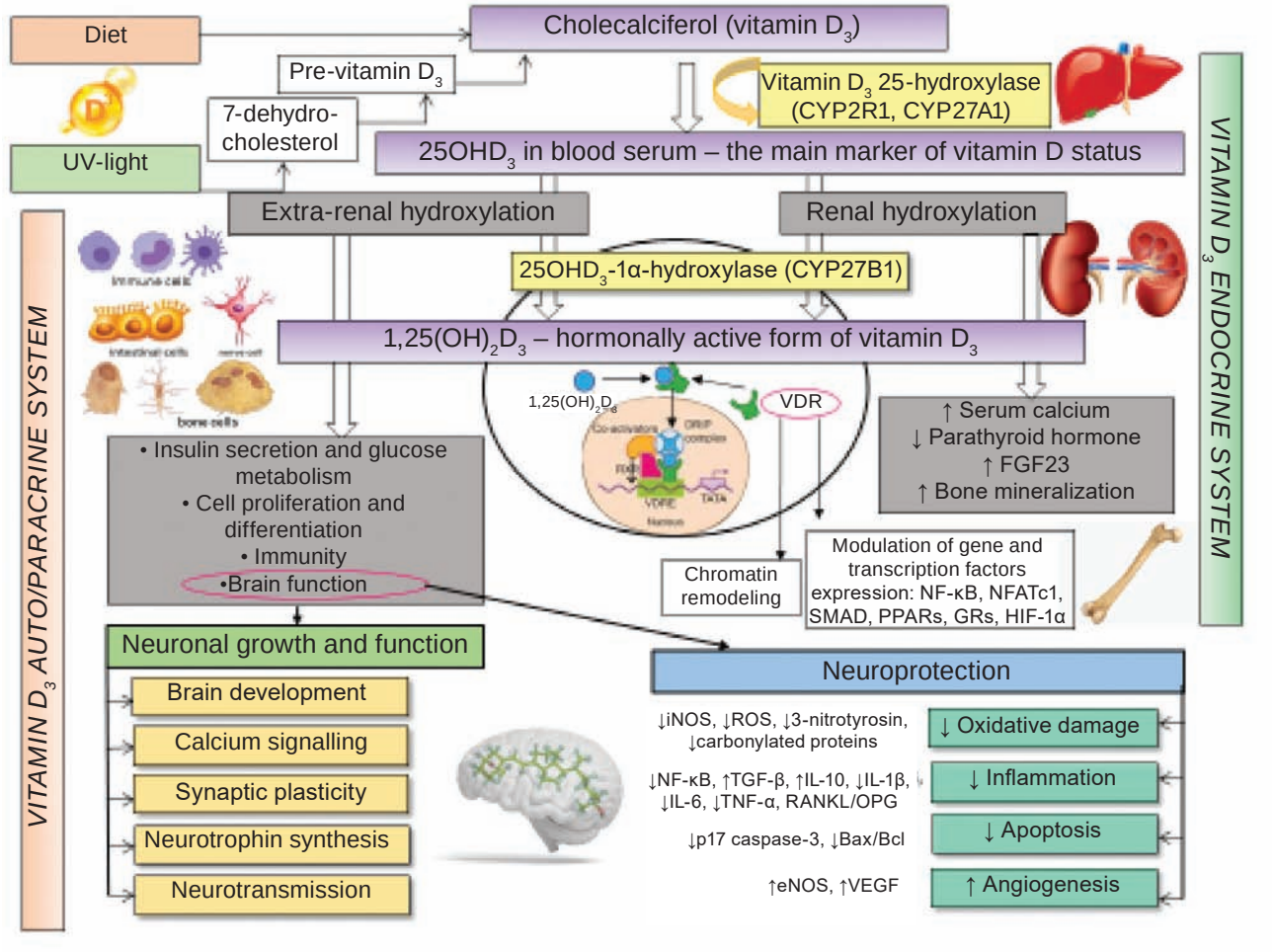

Fig. 1. Involvement of vitamin D_3 auto-/para-/endocrine system in the regulation of cellular functions. $1\alpha,25(OH)_2D_3$ – 1,25-dihydrocholecalciferol; $25(OH)D_3$ – 25-hydroxyvitamin D_3; CYP – cytochrome P450; RXR – retinoid X receptor; VDR – vitamin D receptor; VDRE – vitamin D responsive element; GR – glucocorticoid receptor; FGF23 – fibroblast growth factor 23; ILs – interleukins; NF-κB – nuclear factor kappa B; NFATc1 – nuclear factor of activated T cells c1; SMAD – SMAD transcription factors

min D_3, changes in the functioning of the D_3 auto-/paracrine system and the level of prooxidant processes in body tissues. In a model of osteoporosis induced by dietary deficiency of D_3 or prolonged administration of the synthetic glucocorticoid prednisolone, elevated generation of ROS by isolated hepatocytes, increased oxidative damage to proteins (by the content of carbonyl groups), and accumulation of TBA-reactive lipid peroxidation products were found. In addition, prednisolone elicited a decrease in the activity of key enzymes of the liver antioxidant defense system (SOD, catalase, glutathione peroxidase), while the activity of prooxidant enzymes NAD(P)H-quinone oxidoreductase and semicarbazide-sensitive amine oxidase increased [14-16]. The intensification of free-radical processes was correlated with a higher proportion of necrotic cells among isolated hepatocytes under glucocorticoid load [13].

The negative effect of glucocorticoids (GC) on vitamin D3 metabolism and the state of the D_3-auto/paracrine system in bone tissue can lead to disruption of VDR-mediated osteoblast-osteoclast interaction during bone remodeling. The pro-resorptive signaling pathway of the receptor activator of nuclear factor κB (RANK) and its modulators: RANK ligand (RANKL) and the RANKL decoy receptor – osteoprotegerin (OPG) [17] can be considered as integrating components of such interaction. It has been shown that long-term administration of GC is characterized by a significant decrease in the level of RANK and osteoprotegerin in the bone tissue of rats without significant changes in RANKL, as well as a reduced content of osteocalcin, a marker of bone tissue formation [18, 19]. At the same time, increased proliferation of RANK-expressing osteoclast precursor cells in the bone marrow was observed [20]. The obtained results indicate a simultaneous suppression of both osteoblast-mediated bone formation and osteoclast-activated bone resorption, against the background of vitamin D_3 deficiency and decreased bone VDR expression.

Prednisolone has been shown to suppress the functional activity of nuclear transcription factor κB (NF-κB) in bone tissue, which was accompanied by a decrease in NF-κB-mediated expression of VEGF and iNOS [19]. In addition, prednisolone administration resulted in significant changes in signaling through VDR and PPARγ, as well as in the interaction of two critical signaling axes – HIF-1α/VEGF and RANK/NF-κB, that probably reflects impaired coupling of osteogenesis and angiogenesis. An antiresorptive effect of D_3 and its combined use with methylenebisphosphonate was revealed, as evidenced by partial or complete normalization of VDR-mediated expression of regulatory proteins of the osteokine RANKL/RANK/OPG and vitamin D_3-auto-/paracrine systems in bone tissue [21]. Restoration of D_3 bioavailability and normalization of VDR expression are important for restoring cross-talk between PPARγ and VDR pathways in bone tissue, which controls the functional relationship between the HIF-1α/VEGF and RANK/NF-κB axes (Fig. 1).

Neurodegeneration in glucocorticoid-induced vitamin D_3 deficiency. An additional important area of research for the scientific group led by PhD Ihor Shymanskyi is the study of molecular and cellular mechanisms of neurodegeneration in vitamin D_3-deficient states. It is known that long-term use of glucocorticoids as anti-inflammatory agents increases the risk of developing a number of complications of the central nervous system (CNS). GC-induced neurotoxicity manifests itself in the form of disorders of motor and sensory functions, emotional status and integrative brain functions, such as memory and learning [22]. The current task was to establish the molecular mechanisms of the development of GC-induced neurotoxicity in relation to D_3 deficiency and to assess the therapeutic efficacy of cholecalciferol.

In an experimental model of neurotoxicity caused by long-term administration of prednisolone, significant disturbances in the functioning of the vitamin D_3 auto-/para-/endocrine system were demonstrated. Progressive development of vitamin D_3 deficiency, which was recorded by a decrease in the content of $25(OH)D_3$ in the blood serum and cerebrospinal fluid, was accompanied by elevated mRNA expression and protein content of VDR and CYP27B1 (an enzyme of calcitriol biosynthesis) with a concomitant reduction in the protein expression of the CYP24A1 (calcitriol catabolism enzyme), as well as VDBP in brain tissue [23].

Histological studies revealed pathological changes in the cytoarchitecture in different structural areas of the rat brain. Prednisolone reduced cell density in the CA2 and CA3 sectors of the hippocampus. Pyramidal neurons exhibited an increase in the volume of intact cell nuclei, accompanied by an elevated level of euchromatin, indicating activation of synthetic processes in them. An increase in the cross-sectional area of neurons in layer V of the prefrontal cortex was established, while the cross-

sectional area of neurons in this layer of the sensorymotor cortex decreased. Signs of neurodystrophic changes were found in the ganglion layer of the cerebellum [23].

The established morphometric changes in neurons of different brain areas were accompanied by significant functional disorders: a decrease in K^+-depolarization-induced exocytosis in isolated nerve terminals, inhibition of Ca^{2+}-dependent fusion of vesicles with synaptic membranes under the neurotoxic effect of prednisolone, a decrease in long-term plasticity (LTP) of CA3-CA1 synapses of the hippocampus [23]. Such a decrease may explain the alterations in behavioral response that we identified: suppression of exploratory behavior, an increase in the level of anxiety, and a depressive-like state.

We have shown the accumulation of nitrated proteins, an increase in the intensity of poly-ADP-ribosylation of proteins under the action of prednisolone, a rise of TNF-α, IL-1β, iNOS, TGF-β and VEGF. This indicates a low-gradient inflammatory process in the CNS during GC-induced neurotoxicity [24].

It was established that GC-induced programmed cell death is enhanced in the rat brain through mainly the mitochondrial mechanism [25]. A reliable decrease in the number of NG2+ cells (oligodendrocytes) in the hippocampus was revealed, however, an increase in the number of astroglial cells was observed in various areas of the brain [25].

With the prolonged action of prednisolone, we conducted the first study of the role of NF-κB as a universal transcriptional regulator of cell survival and an integral factor of intercellular communication cytokine systems in the development of neuropathology associated with glucocorticoid load. Prednisolone enhanced the nuclear translocation of the p65 subunit of NF-κB, elevated the content of its phosphorylated forms and transcriptional activation of NF-κB [24, 26]. An increase in the level of RelA (p65)/p50 NF-κB dimers in the cortex, the function of which in neurons is associated mainly with cytotoxicity, and a decrease in c-Rel-containing dimers responsible for cytoprotective effects were established.

One of the least studied pathways of NF-κB activation in the CNS is the signaling pathway involving the RANK/RANKL/OPG cytokine axis. The components of the axis are expressed in CNS cells involved in neuroinflammation, mainly in microglia, as well as in resident macrophages and inflammatory cells capable of crossing the blood-brain barrier [27]. Upon the neurotoxic effect of prednisolone, a decrease in the RANKL level has been shown against the background of an increase in the content of osteoprotegerin and RANK in the nervous tissue. Immunohistochemical labeling of rat brain sections showed an increase in the number of VDR-positive neurons in the cortex and a decrease in the hippocampus under the action of prednisolone.

We demonstrated an increase in glucocorticoid and mineralocorticoid level ratio (GR/MR) due to elevated GR content and reduced MR content in the nervous tissue that was consistent with vitamin D_3 insufficiency [28].

Therapeutic administration of D_3 partially corrected the damage to the rat brain caused by prolonged exposure to prednisolone. The positive effect of D_3 in GC-induced neurotoxicity is closely related to its neuroprotective effect in the CNS as a neurosteroid hormone.

Vitamin D_3 deficiency and neurodegeneration in diabetes mellitus. Diabetic neuropathy is one of the common complications of diabetes mellitus (DM) of both types 1 and 2, and neurological disorders affect all segments of the nervous system: the brain and spinal cord, the peripheral and autonomic nervous system.

In experimental T2DM, we found a significant drop in the level of blood serum $25(OH)D_3$ that correlated with a deficiency of this metabolite in brain tissue. Insufficient D_3-vitamin status of rats with T2DM paralleled an increase in the mRNA and protein levels of both CYP27B1 and CYP24A1, which respectively provide local formation and degradation in the nervous tissue of the hormonally active form of D_3. At the same time, a significant decrease in the content of VDBP in the nervous tissue was shown in T2DM. In addition, diabetes led to a moderate upregulation of the VDR protein expression, through which the auto/paracrine effects of $1\alpha,25(OH)_2D_3$ in the brain are realized [29].

We showed an increased advanced glycation end products (AGEs) content in the brain of diabetic animals was accompanied by accumulation of nitrated proteins (by 3-nitrotyrosine level) as well as proinflammatory cytokines TNF-α and interleukin 1β. It was also found intensification of protein pol-ADP-ribosylation in brain cell nuclei [30]. An elevated content of the brain p17 caspase-3 fragment and proapoptotic protein Bax was found, while the antiapoptotic protein Bcl-2 decreased.

Using Western blot analysis and real-time PCR, a diabetes-elicited increase in the expression levels of both NF-κB transcription factor and mRNA of its large subunit p65 NF-κB in rat brain tissue was established. The transcriptional activity of NF-κB/p65, detected by the immunoenzyme method by the level of its binding to a double-stranded DNA fragment containing an NF-κB response element, was also raised in the cell nuclei of the brain of diabetic animals. At the same time, a downregulation of the inhibitory protein IκB-α expression and an increase in the NF-κB(p65)/IκBα ratio indicating the recruitment of the canonical NF-κB pathway in the development of neurodegeneration in type 2 diabetes were observed.

Overall, the results of the studies on the selected models indicate a close relationship between the functional activity of key VDR-dependent and NF-κB-associated cellular signaling pathways and the level of vitamin D_3 bioavailability for the central nervous system. Disruptions in the functioning of regulatory networks due to vitamin D_3 deficiency of various etiologies, at least partially, can cause the development of prooxidant, proinflammatory and proapoptotic processes in liver pathology, diseased bone tissue (osteoporosis) and neurodegeneration in the brain.

Therapeutic mechanisms of vitamin D_3 action in pathological conditions. Since the pleiotropic effects of the hormonally active form of vitamin D_3 are realized in target tissues due to VDR-mediated gene transcription, restoration of the D_3 vitamin status of the body is a necessary prerequisite for the effective correction of the identified disorders in the pathological conditions under study (dietary and glucocorticoid-induced D_3 deficiency, type 2 diabetes mellitus). First of all, we revealed the ability of exogenous vitamin D_3 to normalize the level of its bioavailability and the content of vitamin D_3-auto-/para-/endocrine system components in vitamin deficiency of various etiologies [10-13, 23, 29].

With normalization of vitamin D_3 status, manifestations of oxidative-nitrosative and genotoxic stress, the content of nitrated and poly-ADP-ribosylated proteins significantly decreased, the effectiveness of the antioxidant defense system increased [13-15, 23, 29], the synthesis of pro-inflammatory factors (TNF-α, IL-1β, iNOS, and VEGF) was reduced, and programmed cell death in nervous tissue was inhibited [13, 23]. In GC-induced neurotoxicity and diabetes mellitus, vitamin D_3 suppressed the release of neurospecific markers of brain damage into the blood, such as NF-L (neurofilament light chain), astroglial proteins S100B (Ca^{2+}-binding/sensor protein) and GFAP (glial fibrillary acidic protein) and MBP (myelin basic protein), characterizing the state of demyelination/remyelination of axons.

We have demonstrated the high efficiency of D_3, mediated by calcitriol, in suppressing the canonical NF-κB activation pathway [13, 20, 24] in particular through the effect of D_3 on the expression of components of the RANK/RANKL/OPG signaling triad. Inhibition of NF-κB activity may be associated with a decrease in the production of interleukins, tumor necrosis factor, angiotensinogen, plasminogen activator inhibitor, etc., the synthesis of which is well established to be induced during inflammation and mediates it (Fig. 1).

The significant therapeutic potential of vitamin D_3 as a natural VDR ligand is observed in inflammatory and autoimmune processes (rheumatoid arthritis), in osteoporosis (alimentary, postmenopausal and steroid-induced osteoporosis), cardiovascular diseases, cancer (prostate, small intestine, breast cancer, myelodysplasia, leukemia) [31], dermatological (psoriasis, actinic keratosis, seborrheic dermatitis) and nervous diseases [18, 31-33]. Accordingly, vitamin D_3 deficiency is considered a universal risk factor for the development of multifactorial pathologies [34], and its management may become a promising pharmacotherapeutic approach.

Neuroprotective role of vitamin B_3 (nicotinamide) in diabetes-associated nervous system disorders

The role of vitamin B_3 in cellular metabolism. Vitamin B_3 comprises three biologically active forms: nicotinic acid (niacin), nicotinamide (NAm), and nicotinamide riboside (NR). All serve as precursors for the biosynthesis of nicotinamide adenine dinucleotide (NAD^+), a key multifunctional factor involved in cellular metabolism and energy homeostasis. NAD^+ also serves as a precursor for nicotinamide adenine dinucleotide phosphate ($NADP^+$) and their reduced forms, NADH and NADPH. In metabolic pathways such as glycolysis and the citric acid cycle, NAD^+ serves as an electron carrier in redox reactions. NADH in mitochondria transports electrons to the electron transport chain NAD^+/NADH ratio is a key indicator of the cellular redox state and a potential biomarker of oxidative stress. Intermediates in NAD^+ biosynthesis, such as NR and nicotina-

mide mononucleotide (NMN), have gained attention for their role in elevating NAD^+ levels, potentially improving metabolic processes, enhancing physical endurance, and protecting against age-related cellular decline [35]. An important area of research, directed by Professor Tamara Kuchmerovska, aimed to elucidate the mechanisms of non-coenzyme actions of vitamin B_3 and NAD^+ in the nervous system. One of the non-coenzyme functions of NAD^+ includes its role as a substrate for a key enzyme in protein ribosylation processes, poly(ADP-ribose) polymerase-1 (PARP-1) [30].

Neuroprotective action of NAD^+ in diabetes mellitus. Diabetic peripheral neuropathy is a common chronic complication of both T1DM and T2DM, characterized by a distal-to-proximal loss of nerve function [36, 37].

Post-translational modifications of proteins, particularly poly-ADP-ribosylation mediated by poly(ADP-ribose)polymerases (PARPs), a diverse family of 18 proteins, play a crucial role in diabetes and its complications (Fig. 2).

While essential for genomic integrity, excessive PARP-1 activation in diabetes leads to NAD^+ depletion, ATP loss, and increased cell death. Our previous studies demonstrated that PARP-1 overactivation in brain cell nuclei contributes to the development of diabetic neuropathy. In experimental T1DM, it was established that increased brain and liver DNA damage is accompanied by the upregulation of PARP-1 level and activity, poly-ADP-ribosylation of nuclear proteins, reduction of NAD^+ and ATP levels, and enhancement of apoptotic markers such as the 89 kDa PARP-1 cleavage fragment [30, 38]. Administration of NAm, a PARP-1 inhibitor, regulated diabetes-induced alterations in the brain at the molecular level by reducing DNA damage and PARP-1 activity. This effect is likely mediated by both direct competitive inhibition of PARP-1 and the antioxidative properties of NAm. Additionally, NAm, by activating urea cycle enzymes in the livers of diabetic rats, enhanced ammonia detoxification that attenuates the negative effects of ammonia on the brain [39].

To further evaluate PARP-1 inhibition for the prevention of PARP-1 overactivation and inflammatory processes in experimental T1DM, we assessed the effects of structurally diverse PARP-1 inhibitors: NAm, a natural PARP-1 antagonist, and the synthetic PARP-1 inhibitor 1,5-isoquinolinediol (Iso). Both inhibitors prevented brain cell death by inhibiting the activation and fragmentation of PARP-1, reduced inflammatory markers by decreasing the content of IL-4 and monocyte chemotactic protein-1 in blood serum. In contrast to Iso, NAm has shown greater efficacy in protecting cells against excessive depletion of cell energy reserves [38]. We demonstrated *in vitro* that NAm exerts cytoprotective and antioxidant effects against streptozotocin-induced oxidative stress in isolated rat pancreatic cells [40].

Involvement of vitamin B_3 in the prevention of the diabetic retinopathy development. Diabetic retinopathy (DR) is a leading cause of vision impairment and blindness in diabetic patients. Its pathogenesis, associated with inflammation, involves complex interactions between neurons, glial cells, and vascular components of the retina [41]. Our studies have revealed a link between PARP-1 acti-

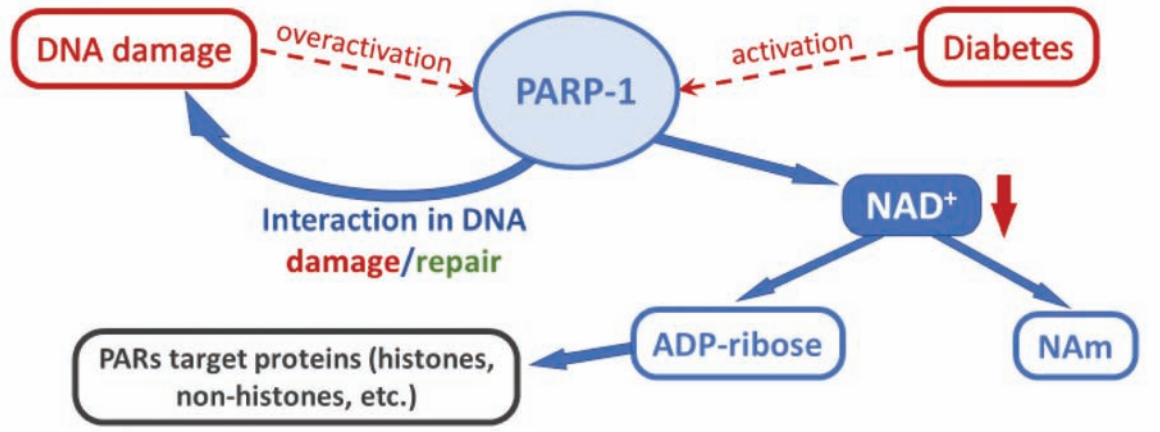

Fig. 2. NAD^+-mediated neuroprotective mechanism involving PARP-1 in the brain and retina under diabetic conditions

vation and reactive gliosis in DR. Elevated PARP-1 expression, increased amount of poly(ADP-ribosyl) ated proteins (PARs), and reactive gliosis markers were observed in diabetic retinas [42]. Administration of NAm or 3-aminobenzamide (3-AB) significantly reduced gliosis by lowering levels of PARP-1, PARs and GFAP and inhibiting the formation of PARP-1 and GFAP cleavage products, as well as by partial restoration of retinal angiostatin levels, indicating the potential of the latter as retinoprotective agents.

The histone deacetylases SIRT1 and SIRT2 play a significant role in neurodegenerative disorders [43]. Similar to PARP-1, activation of these enzymes can lead to depletion of the NAD^+ pool in cells. Immunoblotting analysis revealed alterations in NAD-dependent histone deacetylases in T1DM, specifically a decrease in SIRT1 levels and a more than threefold increase in SIRT2 compared to control animals [44]. These changes may contribute to the development of diabetic encephalopathy. Interestingly, the significant upregulation of SIRT2 may serve as an early marker of neurodegenerative processes, aligning with findings from other studies [45]. Our research group investigated the ability of PARP-1 inhibitors, such as NAm or Iso, to modulate the diabetes-induced alterations in SIRT1 and SIRT2 expression to prevent brain dysfunctions. Nicotinamide stimulated SIRT1 expression in the brain nuclei of T1DM rats, probably through PARP-1 inhibition in mitochondria [46]. However, SIRT2 overexpression may be a result of DNA damage induced by T1DM, as SIRT2 has both deacetylase and mono-ADP-ribosyltransferase activities [44]. It should be noted that Iso did not significantly affect SIRT1 or SIRT2 expression under these conditions, while NAm can support mitochondrial biogenesis by replenishing NAD^+ and enhancing ATP synthesis. Moreover, administration of NAm to diabetic rats significantly increased the NAD^+/NADH ratio, a key regulator of brain metabolic processes. Our results confirm the neuroprotective role of vitamin B_3 at the molecular level mediated by the inhibition of SIRT2 expression and the activation of SIRT1, which enhances post-translational modifications of histone and non-histone proteins in response to diabetes-induced neuronal stress [44]. Thus, sirtuins may be promising therapeutic targets in the treatment of neurodegenerative diseases particularly with novel potent SIRT2 inhibitors.

Neuroprotective effects of nicotinoyl-GABA in diabetes mellitus. Gamma-aminobutyric acid (GABA), the basic inhibitory neurotransmitter in the CNS, plays a crucial role in reducing neuronal excitability and preventing overstimulation. Diabetes disrupts GABAergic function in both type 1 and type 2 diabetes, contributing to impaired blood glucose control, cognitive dysfunction, peripheral neuropathy, energy homeostasis disturbances, and oxidative stress-induced neuronal damage [47-49]. We explored the potential neuroprotective effects of nicotinoyl-GABA (N-GABA, or pikamilone), a synthetic compound combining nicotinic acid and GABA. Our experimental findings provide the first evidence that the neuroprotective effects of NAm and N-GABA in the CNS may be mediated through their ability to modulate apoptosis regulators, specifically by reducing the levels of Bax and NF-κB p65 subunit. Additionally, NAm and N-GABA enhance the expression of angiogenesis-related proteins, such as VEGF and nNOS, in the brains of diabetic rats. At the same time, these compounds reduce the levels of the astroglial marker GFAP and the neuroaxonal damage marker Nf-L in blood serum, likely due to their ability to reduce blood-brain barrier (BBB) permeability. This contributes to the preservation of BBB structural integrity and improves the metabolic capacity of the diabetic brain [50].

Thus, NAm or N-GABA, a conjugate of vitamin B_3 and GABA, combines the neuroprotective properties of both molecules, enhancing nervous system functions and alleviating symptoms of diabetic neuropathy (Fig. 3).

Peripheral neuropathy is one of the most common diabetes complications. Previously, we demonstrated that experimental diabetic peripheral neuropathy is associated with sciatic nerve oxidative/nitrosative stress, nerve conduction deficits, and small sensory nerve fiber dysfunctions, all of which were alleviated by baicalein administration [51]. It is well known that myelin basic protein (MBP) is a key structural component of the myelin sheath, essential for maintaining nerve fiber integrity and function. Our findings demonstrated that MBP levels are reduced in the brains of diabetic rats. However, administration of NAm or, more effectively, N-GABA significantly increased MBP expression, enhancing myelination, nerve conduction velocity, and overall axonal integrity. These effects were accompanied by the inhibition of the polyol pathway of glucose metabolism and by reduced sorbitol accumulation in the sciatic nerves, restoring their morphometric parameters. Decrease in primary sciatic nerve de-

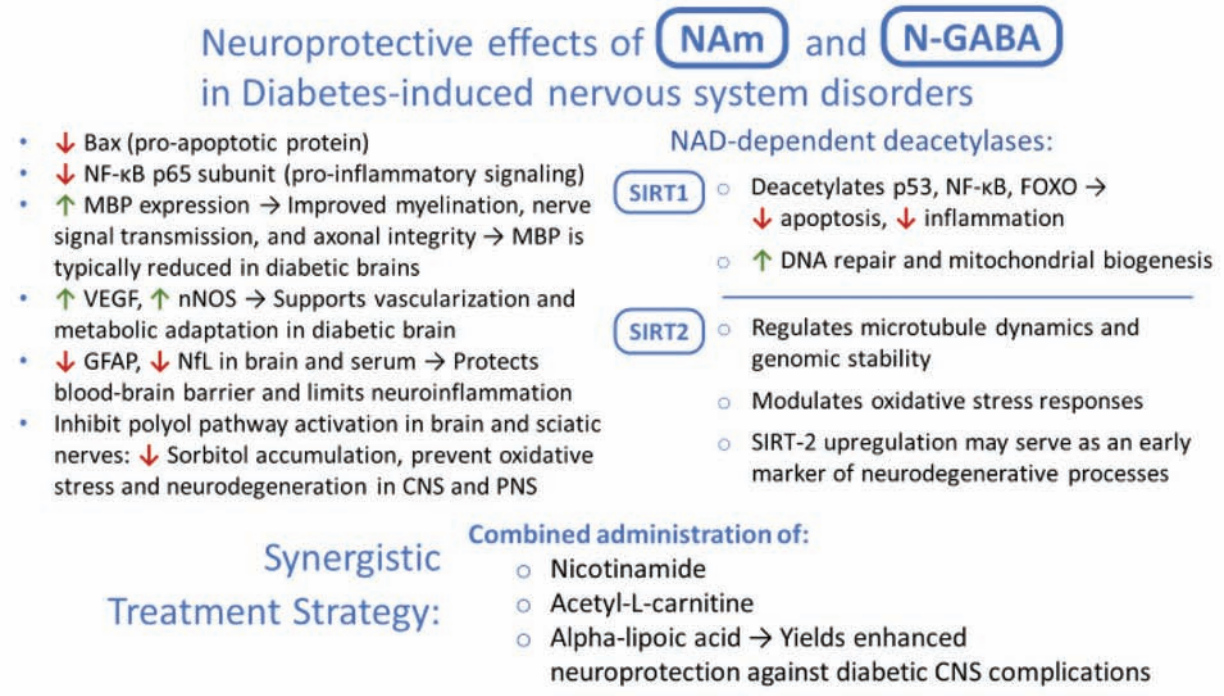

Fig. 3. Protective action of NAm and N-GABA on the content of functionally important brain proteins and NAD-dependent deacetylases in nervous system disorders caused by diabetes mellitus

generation prevented the progression of diabetic encephalopathy and peripheral neuropathy [52]. These results support the therapeutic potential of NAm and N-GABA in preventing the development of nerve cell dysfunction caused by T1DM through neuroprotective effects on both the central and peripheral nervous system, and the investigated proteins may serve as biomarkers for diabetes-induced CNS alterations and potential therapeutic targets.

Our previous studies also demonstrated that 1-methylnicotinamide (MNA), a derivative of NAm methylated by nicotinamide N-methyltransferase (NNMT), exerts neuroprotective effects against diabetes-induced CNS impairments by modulating the serotonergic system, maintaining ion gradients, partially normalizing synaptosomal Na^+/K^+-ATPase activity, and increasing NAD^+ levels through NNMT inhibition [53]. Our findings also indicate that vitamin B_3, its derivatives, and their combinations with other naturally occurring, non-toxic compounds may exhibit synergistic effects when used in combination, compared to monotherapy. We demonstrated that a combination of NAm, acetyl-L-carnitine, and alpha-lipoic acid significantly improved CNS functions in T1DM through modulation of ion gradients, normalization of NAD^+ and ATP levels, and reduction of sorbitol accumulation in the brain [54]. Vitamin D_3 deficiency facilitates an imbalance between the processes of excitation/inhibition of the glutamate/GABAergic systems of the brain and is accompanied by the development of neuroinflammation [85]. Moreover, our research group demonstrated that vitamin D_3 administration to diabetic rats partially inhibits the mRNA expression of the parp-1 gene and normalizes the mRNA expression of the *bax* gene in the heart [86].

Collectively, the results of our studies confirm at the molecular level the neuroprotective potential of vitamin B_3 and its derivatives in diabetes mellitus. Both individually and in combination with other biologically active compounds, they may prevent the development of neurodegenerative and other pathological processes. Future studies should focus on the development of new antidiabetic and neuroprotective agents based on vitamin B_3.

Vitamin B_1 (thiamine): metabolism and neurotropic action in neurodegenerative processes

The relevance of the problem of vitamin B_1 deficiency. The scientific direction of studying the metabolism and regulatory properties of vitamin B_1 (thiamine) as a specialized area of vitamology was established through the studies of Ukrainian bio-

chemists, initiated by Academician O. V. Palladin [1, 2]. The basis for the development of this branch of science was the study of the chemical structure, metabolism and biological activity of thiamine and its derivatives [55]. In particular, the presence of three phosphorus esters of thiamine in all living cells has already been established: thiamine monophosphate (ThMP), thiamine diphosphate (ThDP) and thiamine triphosphate (ThTP). ThDP has been shown to be involved in cellular metabolism as a coenzyme, including its role in the oxidation of pyruvate and other keto acids.

Many conditions have been described that are associated with thiamine deficiency and may lead to the development of Wernicke's disease [56], neurodegenerative diseases are associated with thiamine deficiency [57], cognitive function disorders in adults [58], nervous system disorders in children [59].

It becomes clear from the above that only the identification of the molecular mechanisms underlying the close link between thiamine deficiency and the development of neurodegenerative processes can help in the search for ways of overcoming the specified disorders. Current systematic research on the metabolism and biochemical functions of vitamin B_1 is being conducted in the Department under the leadership of Dr. Sci. Yulia Parkhomenko.

Regulation of vitamin B_1 metabolism and accumulating evidence for non-enzymatic mechanisms of its action. The first studies carried out by the Vitamin Laboratory in the field of thiamine biochemistry were devoted to studying the distribution of thiamine and its phosphorylated derivatives in animal tissues under various conditions [60].

The possibility of using radioactively labeled thiamine (^{35}S-thiamine) in experiments has opened up new perspectives in the study of thiamine metabolism. The researchers administered ^{35}S-thiamine to rats and then determined the distribution of the label among thiamine derivatives in tissue extracts. It was found that as early as 1 h after administration of ^{35}S-thiamine to rats, most of the label was detected in the ThTP fraction, but not in the ThDP fraction. Quantitatively, it represented more than 80% of the total thiamine phosphate pool [61].

The results obtained were a significant achievement, as they were the first and indisputable evidence that ThTP has the highest exchangeability rate among all thiamine phosphates. This observation was later confirmed in experiments involving the incorporation of $^{32}P_i$ from ATP into thiamine phosphates [62].

Further studies on the biochemistry of thiamine focused on investigating and clarifying the mechanisms of involvement of vitamin B_1 and its derivatives in the regulation of cellular processes [63, 64]. And because these events extended beyond the coenzymic functions of vitamin B_1 as a precursor of the coenzyme ThDP, the term "non-coenzymic mechanisms of action" has become customary to describe scientific results in this field.

Over the next few years, we achieved two significant results: (1) a new method for the determination of thiamine phosphate esters in biological material was developed; (2) the involvement of thiamine phosphates in the regulation of the pyruvate dehydrogenase (PDC) complex by a non-coenzymic mechanism was demonstrated. We proposed using cation exchange resins instead of anion exchange resins for the separation of thiamine phosphates in biological samples. This allowed, using the positive charge of the molecules, to achieve a clear and effective separation of ThTP from ThDP, which had not been done before. An article describing the method was published in 1976 in the Ukrainian Biochemical Journal, and in 1979, in Methods In Enzymology (Academic Press) [65].

The identification of the non-coenzyme role of thiamine phosphates in the regulation of PDC yielded unexpected results. While dynamically studying the effect of exogenous thiamine on the activity of ThDP-dependent enzymes in the livers of rats with intensified lipogenesis, instead of the expected upregulation of PDC activity in the first hours after thiamine administration, we observed a reliable downregulation along with an increase in both the ThTP content and the ThTP/ThDP ratio. The studies were continued on isolated mitochondria by modulating the dynamics of thiamine or ThDP uptake. It was assumed that ThTP and ThDP can affect PDC activity by interacting with its regulatory enzymes – PDC kinase and phosphatase. It was demonstrated that the interaction of ThDP with the regulatory enzymes of PDC always leads to activation of the complex. At the same time, the interaction of ThTP with the PDH phosphatase causes inhibition of the activity of PDH phosphatase and, accordingly, a decrease in the activity of PDC. Thus, the possibility of the regulatory effect of thiamine derivatives on the activity of the multienzyme pyruvate dehydrogenase complex was clearly demonstrated [66].

The data obtained are important for understanding the mechanism of vitamin B_1 action on

the synthesis of the neurotransmitter acetylcholine (ACh).

Based on the obtained results regarding relationship between thiamine and ACh metabolism, a hypothesis was postulated suggesting the existence in nerve cells, in addition to the ThDP pool, which binds to enzyme proteins, also of a rapidly exchanging pool, associated with the function of the excitatory membrane and ACh turnover [67-69].

Thiamine-binding proteins (TBP). In the late 20th and early 21st centuries, proteins involved in thiamine metabolism [69] and thiamine-binding proteins [70, 71] were the subject of careful investigation. The study of thiamine-binding proteins was considered one of the most effective approaches to identify new non-coenzyme effects of vitamin B_1 in cells, and this direction remains a priority today. Using an affinity sorbent with thiamine as a ligand synthesised in collaboration with Dr. A. V. Volk, we developed a method to isolate thiamine-binding protein (TBP) from the brain [70] and studied its properties [72]. It was shown that TBP is present in the plasma membrane of cells from all tissues studied (liver, kidney, brain) and that TBP from different tissues has similar properties. We demonstrated the presence of two separate active centres on the thiamine-binding protein, responsible for thiamine binding and ThTP hydrolysis. The localisation of the active centre of this protein, which exhibits thiamine triphosphatase activity, on the inner surface of the plasma membrane has been confirmed [73].

When studying the properties of TBP its molecular weight (about 100 kDa) and the presence of at least two subunits were established. It was shown that TBP selectively hydrolyzes thiamine phosphates, and most actively – ThTP. The affinity of TBP for ThTP is strictly selective among di- and trinucleotides [74].

All studies on the properties of TBP did not allow identifying it with any of the known thiamine transporters. The protein was therefore forgotten for a while, but not for long. The question of its identification became relevant when researchers were asked to identify and study brain proteins with a high affinity for the thiamine molecule [75]. Using affinity chromatography, spectrophotometry to determine enzymatic activity, mass spectrometry, western blotting, amino acid analysis, and bioinformatics it was the substantiated assumption that the previously isolated TBP is part of the LRP4-Agrin protein complex, which is a component of the nicotinic acetylcholine receptor (nAChR) cluster [76]. The mutation of any of the residues (ASN1783, ILE1785) eliminates the interaction of Agrin-LRP4 and subsequent clustering of nicotinic AChR. The probability of this assumption is confirmed by using molecular modeling to assess the binding of the thiamine molecule to the specified proteins (Fig. 4). MS analysis showed the presence of the z8-peptide characteristic of the neuronal isoform of Agrin in our samples.

Thiamine deficiency, nerve cell apoptosis and neurodegenerative changes in the brain. Because brain cells are extremely sensitive to thiamine deficiency, we mainly examined changes in brain tissue or cultured nerve cells. The authors' work, carried out using the experimental facilities of the Univer-

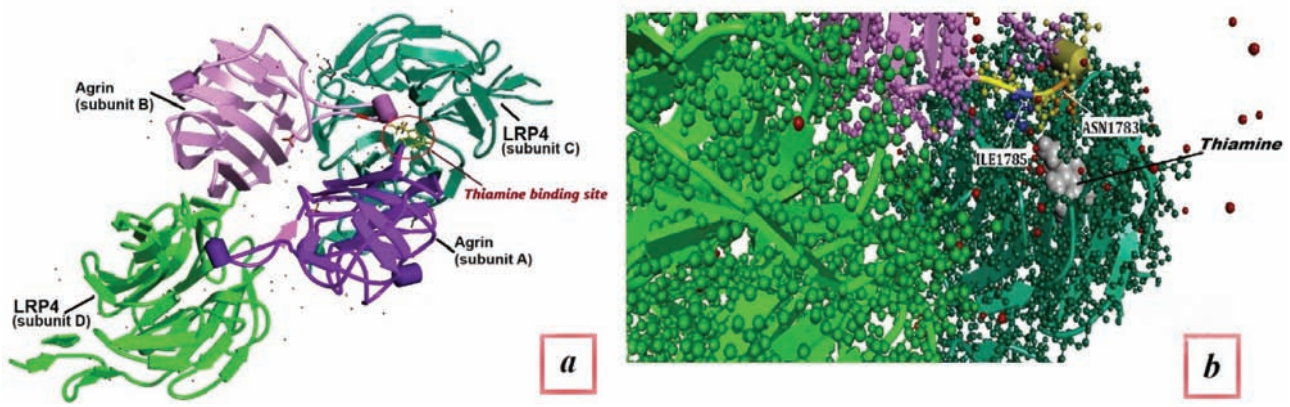

Fig. 4. Representation of 3D model of thiamine interaction with ThBP (protein complex (PDB code 3v64)) using AutoDock Vina and Discovery Studio programmes in the presence of water and other ligands. ***a*** *– High-affinity thiamine binding site;* ***b*** *– high-affinity thiamine binding site on an enlarged scale (subunit A removed), yellow color on subunit B (pink) indicates z8-peptide characteristic of the neuronal isoform of Agrin*

sity of Puerto Rico demonstrated the ability of thiamine antagonists to induce apoptosis in nerve cells as well as the fact that neuronally differentiated cells are much more sensitive to the action of thiamine antagonists than astrocytes [77, 78]. This conclusion is another evidence supporting the proposed hypothesis regarding the mechanisms by which vitamin B_1 participates in the functioning of nerve cells [73]. The conclusion is supported by numerous observations that almost all neurodegenerative diseases are accompanied by vitamin B_1 deficiency and negative changes in its metabolism. Moreover, the recognized experimental models of neurodegeneration are precisely those models of thiamine deficiency, in which we have observed changes in the expression of neurospecific proteins [62, 79].

Accumulation of oxidised forms of thiamine derivatives, oxidative stress and neurodegeneration. Elucidation of the molecular mechanisms by which thiamine deficiency promotes the initiation of neurodegeneration is another important area of our research. It has been shown that alimentary thiamine deficiency against the background of decreased expression of the enzyme thiamine pyrophosphokinase (responsible for the synthesis of ThDP) and the development of oxidative stress in brain tissue leads to the accumulation of the oxidized form of ThDP [64].

Soon after the accident at the Chernobyl Nuclear Power Plant, employees of our department conducted a study on the content of vitamins in the blood of people who suffered from the consequences of the accident and its liquidators. Unfortunately, at that time, no nationwide program of this kind was organized, although the "model" was unique. It was found that, unlike vitamins A and E, the content of which slightly decreased under these conditions, the changes in the content of vitamin B_1 were catastrophic [80]. In fact, an excessive accumulation of the oxidised form of ThDP was observed in the blood of people who received various doses of radiation, due to a critical drop in the content of the cyclic (active) form. Thus, in the blood of some of the liquidators diagnosed with "second stage acute radiation sickness", the cyclic form of ThDP was not detected at all.

Further studies have confirmed that it is oxidative stress and the interaction of thiamine derivatives with free radicals that lead to the described consequences [81]. But how do oxidised forms of thiamine accumulate and why do they not transform further? We did not find an answer to these questions in available scientific publications. We assumed that the thiol form of ThDP can be immobilised by binding to certain cellular proteins, i.e., due to thiamine thiolization of proteins [82]. We believe that thiamintyalization of proteins can initiate neurodegenerative processes in both alimentary thiamine deficiency and other conditions accompanied by oxidative stress, for example, under the influence of unfavorable environmental factors.

Confirmation of the hypothesis by *in vitro* experiments. To confirm the proposed hypothesis, specifically the presence of a mobile pool of thiamine derivatives in cells and the regulatory effect of their components on cellular metabolism through non-coenzyme mechanisms, we conducted experiments with rat brain synaptosomes similar to those described above, but using synthetic thiamine derivatives instead of thiamine. In addition to the well-known coenzyme antagonist of thiamine, oxythiamine (OTh), we used the thiazolium salt decyloxycarbonyl-4-methyl-5-β-hydroxyethylthiazolium chloride (DMHT) in our experiments. This compound features a thiazole ring native to thiamine, as well as derivatives of thiamine and DMHT without the hydroxyethyl radical. The obtained results confirmed our assumptions regarding possible mechanisms of the non-coenzyme effects of vitamin B_1 in nervous tissue [57, 83]. It turned out that the thiamine derivatives used, in the molecules of which there is a thiazole ring characteristic of thiamine, including an unsubstituted hydroxyethyl radical, interacted with thiamine pyrophosphokinase similarly to thiamine and, like thiamine, affected cellular processes by non-coenzyme mechanisms. The results obtained made it possible to explain why pyrithiamine, but not oxythiamine, reproduces neuromuscular transmission disorders characteristic of severe vitamin B_1 deficiency [84]. The fact is that pyrithiamine is a powerful inhibitor of thiamine pyrophosphokinase activity and blocks the synthesis of ThDP, whereas oxythiamine (as well as DMHT in our studies) is a competitive inhibitor similar to the substrate of this enzyme and, forming phosphorylated derivatives like thiamine, it mimics the effects of thiamine on individual cellular processes. That is pyrithiamine blocks the functioning of the mobile pool of thiamine, and oxythiamine creates an alternative pool.

Currently our studies are related to the investigation of the effect of thiamine and its deficiency on the properties of neurospecific proteins, in particular

on the structural organization and expression of proteins in the nerve cell cytoskeleton, as well as those that compose the nicotinic acetylcholine receptor.

*Conclusio*n. In summary, the scientific achievements of the Department of Vitamins and Coenzymes confirm the protective effects of vitamin D_3 (cholecalciferol), B_1 (thiamine) and B_3 (nicotinamide) and their biologically active derivatives under various pathological conditions, in particular neuronal disorders associated with vitamin B_1 deficiency, toxic effects of synthetic glucocorticoids and diabetes melitus (osteoporosis, diabetic retinopathy and neuropathy). The deficiency of these vitamins should be considered one of the key risk factors for the development of multifactorial diseases. Substantiation at the cellular and molecular level of the therapeutic potential of vitamin D_3, B_1 and B_3 in preventing the development of neurodegenerative complications in various pathological conditions opens up additional opportunities for the development of new vitamin supplements.

Conflct of interest. Authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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НАУКОВИЙ ПОСТУП ВІДДІЛУ БІОХІМІЇ ВІТАМІНІВ І КОЕНЗИМІВ ІНСТИТУТУ БІОХІМІЇ ІМ. О. В. ПАЛЛАДІНА НАН УКРАЇНИ НА ШЛЯХУ ДО МОЛЕКУЛЯРНОЇ ВІТАМІНОЛОГІЇ

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Сучасний прогрес молекулярної вітамінології ознаменований переходом на новий рівень більш глибокого розуміння молекулярних механізмів дії вітамінів та їх біологічно активних похідних як високоефективних сполук, що забезпечують контрольовану взаємодію регуляторних систем клітини та метаболічних процесів. Молекулярні механізми плейотропних ефектів гормонально активної форми вітаміну D_3, кальцитріолу ($1\alpha,25(OH)_2D_3$) реалізуються у тканинах-мішенях через рецептори вітаміну D_3 (VDR), які присутні практично у всіх клітинах. Нами досліджно такі VDR-опосередковані ефекти, як модуляція активності факторів транскрипції NF-κB, NFAT, HIF-1, PPAR , залучення регуляторних шляхів за участі HIF-1α/VEGF та RANK/NF-κB, а також сигналювання через глюкокортикоїдні та мінералокортикоїдні рецептори, що відіграють ключову роль в реалізації протиоксидантного, протизапального та протиапоптичного захисту вітаміну D_3 у нормі та за патології (остеопороз, нейодегенеративні порушення, асоційовані з глюкокортикоїд-індукованою нейротоксичністю та цукровим діабетом 2 типу). Досліджено механізми нейротропної дії вітаміну B_3 (нітотинаміду) та похідного нікотинової та аміномасляної кислоти – нікотиноїл-ГАМК (N-GABA). Продемонстровано, що нікотинамід (NAm) гальмує розвиток діабетичної нейропатії шляхом зниження активності та рівня ензиму PARP-1, пригнічення його фрагментації та уникнення пошкоджень DNA у головному мозку, нормалізації рівня протеїнів SIRT1 і SIRT2 в ядрах нервових клітин. Одним із ефективних методичних підходів у наших дослідженнях стало вивчення тіамін-зв’язувальних протеїнів головного мозку та впливу дефіциту тіаміну на стан і експресію нейроспецифічних протеїнів. Сформульовано робочу гіпотезу щодо молекулярних механізмів участі вітаміну B_1 у функціонуванні холінергічної ланки нервової системи, яка передбачає існування в нервових клітинах, крім пулу тіаміндифосфату (ThDP), що зв’язується з ThDP-залежними ензимами, також пулу похідних тіаміну, що швидко обмінюються та спряжені з метаболізмом ацетилхоліну. Наукові здобутки співробітників відділу розкривають терапевтичний потенціал вітамінів D_3, B_3, B_1 та їх біологічно активних похідних у запобіганні розвитку нейродегенеративних ускладнень за різних патологічних станів та обґрунтовують наукові підходи створення нових вітамінних препаратів.

Ключові слова: вітамін D_3, вітамін B_3, нікотиноїл-ГАМК, вітамін B_1, тіаміндифосфат, глюкокортикоїди, фактори транскрипції, нейроспецифічні протеїни, тіамінзв’язувальні

протеїни, оксидативний стрес, запалення, нейродегенерація, цукровий діабет.

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