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# HISTORICAL OVERVIEW OF LIPID BIOCHEMISTRY RESEARCH: FROM INITIAL HYPOTHESES TO UNDERSTANDING THE BIOLOGICAL ROLE OF N-ACYLETHANOLAMINES

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*In the Department of Lipid Biochemistry at the Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine, a systematic approach to studying the functional role of lipids and investigating the lipidome of mammals and humans was developed and implemented from 1988 to 2025. A new class of low-polarity lipids, N-acylethanolamines (NAE), was discovered, and a multifaceted detailed study of their biological activity and functional role in the body was conducted. This allowed the discovery of several new mechanisms for regulating vital processes both in normal conditions and in various pathological states. The relevance of these studies lies in the fact that they not only deepened fundamental knowledge in human and animal biology but also led to the development of several pharmacological agents for the therapy of a range of pathological conditions. The drugs are proposed for use in cardiovascular diseases, allergies, burns, type I and II diabetes, inflammatory processes, oncological diseases, organ transplantation, as well as chronic and acute stress, drug addiction, alcoholism, and post-traumatic stress disorder. Additionally, antiviral agents have been developed that are highly effective against influenza virus, hepatitis C virus, herpes simplex virus, and coronavirus. These agents have no side effects and are protected by 19 Ukrainian patents. The scientific results of the Department of Lipid Biochemistry have been published in over 200 scientific papers and presented at more than 130 international and domestic scientific forums. This article provides a brief review of the main achievements of the Department of Lipid Biochemistry in investigating the biological effects of NAE.*

*K e y w o r d s: lipids, N-acylethanolamines, endocannabinoid system, pathological conditions.*

The Department of Lipid Biochemistry was established at the end of 1980 as initially a cell culture laboratory, which was later reorganized into the Department of Lipid Biochemistry in 1991, headed by Corresponding Member of the National Academy of Sciences and the National Academy of Medical Sciences of Ukraine, Doctor of Biological Sciences, Professor N. M. Hula, and the current head of the department is Candidate of Biological Sciences, Senior Researcher H. V. Kosiakova. In the 1980s and 1990s, the department developed and implemented a systematic approach to studying the functional role of lipids in the body. In collaboration with other scientific groups, particularly from France (led by Professor M. Lagarde, Department of Biochemistry and Pharmacology, INSERM – INSA, Lyon), the USA (Professor E. Berdyshev), New Zealand (Professor M. Vysotsky) and Professor E. Vaskovsky, the methodology for chromatographic analysis of fatty acids, phospholipids, and other complex lipids isolated from biological objects was developed, improved, and standardized. This significant progress enabled the achievement of high reproducibility of results, thereby avoiding artifacts associated with auto-oxidation and lipid decomposition and ultimately obtaining reliable scientific research results. Lipid research is a complex task due to its diversity and structural complexity, and it requires an interdisciplinary approach using various methods of analysis.

As a result of many years of research, the department first discovered a universal pattern of fatty acid imbalance formation under chronic pathological conditions, namely, a paradoxical increase in the

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percentage of polyunsaturated fatty acids, mainly due to arachidonic acid, against the background of elevated total cholesterol levels in biological structures (in patients with arterial hypertension, coronary heart disease, thyroid cancer, etc.) [1, 2].

It was established that the increase in the level of monohydroxylated fatty acids in peripheral blood mononuclear cells of Chornobyl disaster liquidators is a function of the absorbed dose of ionizing radiation and is associated with immune imbalance. Thus, it was in Ukraine that the systematic study of human and laboratory animal lipidomes was first initiated, which preceded the creation of the international lipidome study program for almost two decades. The relevance of these developments lies in the fact that they not only deepened fundamental knowledge in human and animal biology but also led to the development of several pharmacological agents for influencing a range of pathological conditions and the creation of new drugs, contributing to the global scientific community.

It all started in the early 1980s, when under the leadership of N.M. Hula, a minor phospholipid N-acylphosphatidylethanolamine (NAPE), was discovered in the composition of mouse neuroblastoma C1300 N18 cells, which was rarely found in tissues under normal conditions but appeared under pathological conditions. In particular, a group of American scientists from the University of Minnesota, led by Professor H. Schmidt, with whom close cooperation was later established, found this lipid in the infarct zone of the myocardium of dogs with coronary artery occlusion and in the surface layer of the epidermis. It later became known that NAPE is a precursor of a class of minor lipids – N-acylethanolamines (NAE). This fact prompted an active study of the biological activity of NAE. Based on the obtained results, a hypothesis was formulated that the accumulation of NAE in damaged tissues plays a protective role, aiming to minimize myocardial damage under conditions of acute ischemia.

From 1999 to 2024, research by the Department of Lipid Biochemistry was aimed at:

- the role of lipids in the general mechanism of development of pathological conditions and study of the protective effect of natural N-acylethanolamines (1999-2003);
- the mechanisms of protective and adaptive action of long-chain N-acylethanolamines in mammals (2004-2011);
- the effect of N-acylethanolamines (NAE) on growth, proliferation, apoptosis, and tag inclusion in the DNA of transformed cells (2007-2011);
- the antitumor effect of chemotherapeutic drugs under conditions of their delivery by new nanocomposite carriers functionalized with N-acylethanolamines (2010-2014);
- the antiviral activity of N-stearoylethanolamine (NSE) (2012-2024);
- the protective effect of endocannabinoids in metabolic disorders caused by alimentary obesity (2017-2021);
- the protective effect of saturated N-acylethanolamines (NAEs) in various pathological conditions at *in vitro* and *in vivo* studies (2009-2018);
- the mechanisms of geroprotective action of N-stearoylethanolamine (NSE) during normal and pathological aging (2019-2023).

Currently, thanks to the scientific results obtained in the Department of Lipid Biochemistry, a significant step has been taken in understanding the primary mechanisms of biological action of N-acylethanolamines in mammals across various pathological conditions. In this publication, we provide brief overview of the main significant results of these studies from a historical perspective.

## The first results of a study of the biological activity of N-acylethanolamines

The minor lipid class NAE includes acylated ethanolamine derivatives with varying lengths and degrees of acyl chain saturation. In parallel with foreign researchers, the Department's staff showed that the biological activity profile of NAEs depends on the chemical structure of their acyl residue. In the future, the primary focus of the Department's research was NAE with saturated fatty acid chains.

For the first time in the world, employees of the Department of Lipid Biochemistry published a paper

on the antiarrhythmogenic effect of N-palmitoylethanolamine (NPE) in acute myocardial ischemia, accompanied by the restoration of the disturbed lipid composition of heart tissue [3]. NAE has been shown to reduce the level of lysophospholipids, the accumulation of which is associated with the development of arrhythmias in the heart muscle under ischemic conditions. Thus, pre-injection of NPE in rats prevents the accumulation of lysophosphatidylcholine in rat myocardial tissue caused by vasopressin administration, prevents the development of bradycardia and extrasystole, changes in heart rate and modification of the T wave. Subsequently, a stimulating effect on the activity of constitutive NO-synthase (eNOS) of another member of the Class, N-stearoylethanolamine (NSE), was shown, as a result of which blood supply improves and the degree of myocardial damage decreases.

These and other research results suggest that saturated acyl NAE can be used to develop drugs with a cytoprotective mechanism of action. Several important circumstances were taken into account. First, the main N-acyl residues of NAPE (a precursor to NAE synthesis) that accumulate in heart tissue during acute myocardial infarction are palmitate, stearate and oleate, the proportion of which reaches up to 80% in the total structure of NAE. Secondly, NAE is formed in the amounts necessary to detect cardioprotective action only a few hours after acute coronary spasm, and therefore, there is a need to ensure early entry of this compound into the area of myocardial damage, which, obviously, would minimize the degree of irreversible damage to heart muscle cells. In this regard, for the first time in the world, the Department developed the drug containing N-acylethanolamine with saturated acyl chains for the treatment of acute coronary syndrome and ischemic/reperfusion myocardial damage [4-6]. The results of pharmacological studies of NAE under conditions of acute ischemia/reperfusion of the myocardium and liver in rats demonstrated its high efficacy and safety. For example, the $LD_{50}$ for N-palmitoylethanolamine when administered to mice and rats *per os* is more than 5000 mg/kg (Hazard Class IV according to GOST 12.1.007-76).

So, the first drug developed based on NAE was a drug for the treatment of acute coronary syndrome, including acute myocardial infarction, as well as coronary heart disease. The main goal of using the drug is to reduce general and cardiovascular mortality, especially among people of working age.

## N-acylethanolamines in the treatment of morphine and alcohol dependence

In the late 1990s, our research first demonstrated the neuroprotective effect of NAE under conditions of morphine dependence in experimental animals, which is achieved by restoring the content of catecholamines, lipids and free amino acids in rat brain tissue.

A rat morphine dependence model also showed that a mixture of saturated and unsaturated NAE at different course doses reduces the amount of voluntarily consumed morphine. It has been experimentally proven that the most effective course dose is 35 mg/kg, in which rats with morphine dependence reduce the amount of voluntarily consumed morphine by almost 30%. Therefore, a mixture of NAE with saturated and unsaturated hydrocarbon chains may be a potential candidate for drugs with neuroprotective properties for the pharmacotherapy of morphine dependence [7].

Subsequently, in 2014-2016, studies were conducted on the effects of NAE in rats with chronic and acute alcohol poisoning. NSE has been shown to exhibit a pronounced antitoxic, hepatoprotective effect in both acute and chronic alcohol intoxication [8, 9].

## Study of the adaptogenic effects of N-stearoylethanolamine

Today, it is difficult to overestimate the role of the adrenal cortex in the body's response to any form of stress stimuli. Generally, to assess the adaptive reserves of the body under stress, the level of 11-oxycorticosteroids is determined. It is worth noting that various regulatory factors influence the adrenal cortex.

We have shown for the first time that NSE causes changes in the level of 11-oxycorticosteroids (11-OCS) in the blood of experimental animals under various pathological conditions [10, 11]. Thus, when rats are exposed to a dose of 2 Gy, NSE completely prevents changes in the level of 11-OCS in blood plasma. The effect of NSE on the background of immobilization stress leads to an additional increase in the content of 11 OCS. Thus, we found that NSE in the body of experimental animals modulates the level of 11-OCS, thereby affecting the course of the body's stress response. We found that during the action of NAE, in parallel with changes in 11-OCS, changes in the content of adrenocorticotropic hormone in the blood of animals also occur. These results were presented in 2001 in the PhD thesis of one

of the current members of the Lipid Biochemistry Department research group, O. Zhukov [12]. It was hypothesized that the effects of NAE on 11-ACS levels that we found are probably mediated by the hypothalamic-pituitary-adrenal axis.

This hypothesis was tested in studies conducte in 2023 to investigate the effect of NSE on the hormonal balance of the body during normal and pathological aging complicated by a chronic inflammatory process. The effect of NSE on the content of glucocorticoids and the state of the sympatho-adrenal system in rats with normal and complicated chronic inflammatory aging was studied. It was found that in old rats (18-month-old), the content of corticosterone, epinephrine and dehydroepiandrosterone (DHEA) in blood plasma significantly decreased compared to the values in young (4-month-old) rats. The use of NSE in old rats *per os* at a dose of 50 mg/kg of body weight for 10 days causes an increase in the content of corticosterone and DHEA in blood plasma to levels found in young animals but does not affect the level of epinephrine. It was found that the development of a chronic bacterial lipopolysaccharide-induced inflammatory process in old rats causes an even greater decrease in the content of corticosterone, epinephrine and DHEA in the blood plasma of animals. The use of NSE *per os* at a dose of 50 mg/kg of body weight for 10 days in old rats in the process of modeling chronic inflammation in them contributes to an increase in corticosterone and DHEA levels to values characteristic of age-matched controls and increases the epinephrine content to values characteristic of young rats.

When studying the effect of NSE on the state of the hypothalamic-pituitary system of rats in normal and complicated by chronic inflammatory aging, it was found that the content of adrenocorticotropic hormone (ACTH) in blood plasma significantly increases in old rats (18-month-old), compared with its value in young (4-month-old) rats. The oral administration of NSE in elderly rats at a dose of 50 mg/kg of body weight for 10 days results in a significant decrease in ACTH content. We found an even greater increase in the content of ACTH in blood plasma due to the chronic inflammatory process induced by bacterial lipopolysaccharide in old rats. The use of NSE *per os* at a dose of 50 mg/kg of body weight for 10 days in elderly rats in the process of modeling chronic inflammation in them helps reduce ACTH levels to values characteristic of age-related controls. The research results are presented in several publications [13].

## NAE and the nitric oxide (NO) system

Further research on the biological action of NAE and the mechanisms underlying it has resulted in data indicating the involvement of a well-known biological regulator, the signaling molecule nitric oxide (NO), in the cytoprotective mechanisms mediated by NAE. In the studies conducted by the Department, the effect of NSE on the processes of nitric oxide formation in modeling various pathological conditions of the body was investigated for the first time. In a model of ischemic reperfusion injury to rabbit skeletal muscle and ionizing radiation in rats, it was shown that NSE in *in vivo* studies can have a significant effect on the content of stable nitric oxide metabolites in animal muscle tissue and blood. In a model of streptozotocin-induced diabetes mellitus in rats, the ability of NSE to inhibit the activity of the inducible isoform of NO-synthase and activate the constitutive isoform of the enzyme in cells and organs of the cardiovascular system was established. The effect of inhibiting the activity of inducible NO-synthase under the influence of NSE was also observed in *in vitro* experiments on red blood cells obtained from patients with pulmonary hypertension. At the same time, in a model of anaphylactic shock in guinea pigs, NSE administration caused inhibition of constitutive NO-synthase activity and prevented the development of excessive vasodilation [14-16].

Thus, the data obtained indicate the ability of saturated NAE to modulate the formation of nitric oxide in various pathological conditions.

## Anti-allergic and anti-inflammatory effects of NAE

A study of the effect of NAE on the course of delayed and immediate allergic reactions conducted during 2005-2008 showed that NSE slows down the development of allergic reactions (prevents an increase in histamine levels, reduces the activity of constitutive no synthase in the heart of guinea pigs increased by anaphylaxis, normalizes nitrite anion levels), eliminates pro-antioxidant balance disorders (normalizes the content of TBK-active products and the activity of glutathione peroxidase, catalase and superoxide dismutase), and modulates lipid composition of target organ tissues. Moreover, what is extremely important is that the use of NSE significantly increases the survival rate of animals in anaphylactic shock [17].

Subsequently, it was found that the action of NSE also leads to the inhibition of a delayed hyper-

sensitivity reaction and to a dose-dependent inhibition of the activation and proliferation of normal T and B lymphocytes *in vitro* [18].

When studying the effect of NSE on the development of non-specific inflammation during experimental burn in rats, it was shown for the first time that NSE can accelerate the healing process of a thermal burn wound by inhibiting the production of pro-inflammatory cytokines (TNFα, IL-6, IL-1β), normalizing the content of a stable metabolite of nitric oxide-nitrite-anion and the activity of constitutive and inducible NO-synthases, as well as eliminating the imbalance between the processes of free radical lipid oxidation and the activity of antioxidant defense enzymes in plasma, red blood cells, liver, and spleen [19]. The data obtained are presented in a number of scientific publications [19-21] and are protected by a patent [22].

These facts made it possible, for the first time, to characterize NSE as a compound with an anti-inflammatory effect that accelerates burn wound healing processes and decreases the severity of biochemical changes in burn disease.

Thus, by the end of 2012, as a result of our research on the biological effects of NAE, it became clear that these endogenous compounds, if used exogenously, can affect the initial stages of initiation of inflammatory or other processes underlying pathologies or accompanying their course. It was hypothesized that exogenous NAEs mainly act through a preventive mechanism – they prevent the inflammatory process from becoming uncontrolled, which can occur via a receptor mechanism. Cases where exogenous NAE inhibits an already developed inflammatory process, according to our assumption, can also occur by a non-receptor mechanism. It was interesting to note that in healthy control animals, NAE did not affect any biochemical parameter. There was a hypothesis that NAE acts on those biological targets that appear or are activated during the development of a specific pathological process.

Therefore, it became necessary to further focus on elucidating the subtle biochemical mechanisms underlying the biological action of NAE.

## NAE as components of the endocannabinoid system

At the beginning of the study, it was not known how NAEs exert their biological effect – either by direct membranotropic action or by binding to specific receptors on the cell membrane. First, the Department obtained results indicating the direct membranotropic effect of these compounds. In cooperation with the staff of the Department of Muscle Biochemistry (headed by Academician of the National Academy of Sciences of Ukraine, Professor S. O. Kosterin), results were obtained on the ability of NPE to directly and selectively simulate the activity of membrane-bound $Ca^{2+}$-transport systems in cells. It was found that NPE at a concentration of 10 μmol/l stimulates the activity of $Mg^{2+}$,ATP-dependent calcium pump located in the plasma membrane of myometrial cells. At the same time, NPE does not have an activating effect on the $Ca^{2+}$,$Mg^{2+}$-ATP solubilized from the plasma membrane of uterine smooth muscle cells, which was purified using the method of affinity chromatography on calmodulin-sepharose 4B. It was also shown that 10 μmol/l NPE inhibited the energy-dependent accumulation of $Ca^{2+}$ in mitochondria, which is insensitive to the action of ruthenium red, and thapsigargin-sensitive, oxalate-stimulated $Mg^{2+}$,ATP-dependent accumulation of $Ca^{2+}$ in the sarcoplasmic reticulum. It was suggested that the activating effect of NPE on $Mg^{2+}$,ATP-dependent accumulation of $Ca^{2+}$ in the vesicles of the plasma membrane is associated with the accumulation of lyso-phosphatidylcholine in it, and the inhibitory effect of NPE on $Mg^{2+}$,ATP-dependent accumulation of $Ca^{2+}$ in the sarcoplasmic reticulum and mitochondria with predominant accumulation of phosphatidylcholine and sphingomyelin in their membranes [23].

NAE has also been shown to inhibit the entry of monovalent cations through veratridine-activated sodium channels. Subsequently, in 2013, data were obtained that allowed us to hypothesize that such effects of NAE may be due to its influence on $Na^{+}$ and $Ca^{2+}$ channels, and thus, on electrical excitability and membrane currents in cardiomyocytes [24].

The results of these studies made it possible to understand the biochemical mechanism of NAE's effect on myocardial contractile activity, but did not answer the question of how the lipid composition of cardiomyocytes is modified.

In 1988, the group W.A. Devane receptors activated by the psychoactive component of Cannabis sativa, tetrahydrocannabinol, were discovered on the plasma membrane of neurons, which were called cannabinoid receptors (CB), and already in 1992 an endogenous ligand of these receptors, N-arachidonoylethanolamine (anandamide), was identified, which, as it turned out, belongs to the NAE class

by chemical structure. It was logical to assume that other members of this class could also activate CB receptors. Thus, there was evidence that long-chain NAES with polyunsaturated acyl residues are able to activate CB receptors. In contrast, the data for NAEs with saturated acyl residues were contradictory, but it was later recognized that they do not activate CB receptors. In 1995, the term "endocannabinoids" was proposed, which referred to all endogenous ligands of cannabinoid receptors. It was also found that both saturated and unsaturated NAES are synthesized and metabolized in cells along the same biochemical pathways. Later, the results of the Department's research showed that NAES with saturated acyl groups exhibit a cannabimimetic effect (they have effects similar to endocannabinoids), although they do not activate CB receptors. It turned out that NAE with a saturated acyl group can have a regulatory effect on the activity of one of the enzymes involved in endocannabinoid degradation – fatty acid amidohydrolase – and thus affect endogenous levels of anandamide.

Today, the concept of the body's endocannabinoid system, which encompasses CB receptors, their endogenous ligands, enzymes for the synthesis and degradation of these ligands, and compounds with cannabimimetic effects, has been finally established. It is known that endocannabinoids are involved in the implementation of various physiological functions and exhibit a wide range of biological activities; in particular, they are characterized by anti-inflammatory, antioxidant, membrane-stabilizing, immunomodulatory, neuroprotective and adaptogenic effects. Numerous human diseases are associated with dysregulation of the endocannabinoid system; therefore, its pharmacological modulation is a promising strategy for treating various inflammatory, neurodegenerative, cardiovascular diseases, metabolic disorders, ischemia/reperfusion injuries, and cancer.

Endocannabinoids play a crucial role in energy metabolism in peripheral tissues. Adipose tissue metabolism and hormone secretion directly depend on the activation and inhibition of the endocannabinoid system. Stimulation of adipocytes with CB1 receptor agonists leads to the accumulation of lipids in fat droplets, a decrease in the concentration of adiponectin, PPAR-gamma activity, and an increase in lipoprotein lipase activity. Consequently, the cannabinoid system is involved in the pathogenesis of Type II diabetes mellitus. Therefore, regulation of the activity of the cannabinoid system may be a target for therapeutic effects in the treatment of this polyfactorial disease.

In choosing the strategy of our research, the key point was that, according to the latest data at that time, NAEs are very important components of the endocannabinoid regulatory system of mammals; they are able to indirectly affect the activity of other links of the endocannabinoid signaling system through the so-called "Entourage effect" (the effect of the environment). However, even by modulating the state of the endocannabinoid system, it is still impossible to fully explain the biological effects of saturated NAE.

Therefore, in 2014, research began to investigate the mechanisms of biological action of NAE as a component of the body's endocannabinoid system, a process that continues to this day. Let us briefly focus on the results achieved.

### Study of the effect of NSE on insulin resistance and experimental cognitive impairment in mammals

Earlier, in 2008-2009, we obtained results indicating the ability of NAE to influence the course of Type I and Type II diabetes in rats [25]. As a result of studies conducted from 2014 to 2018, the protective effect of N-stearoylethanolamine (NSE) on insulin resistance and experimental cognitive impairment in mammals was experimentally demonstrated and justified for the first time. In particular, it has been shown that the use of NSE in rats restores the content of anionic phospholipids involved in the regulation of the insulin signaling cascade in the structure of the membranes of insulin-dependent tissues and, thus, promotes the flow of glucose into the cells of these tissues, that is, it has a glucose-lowering effect. Administration of NSE contributes to the normalization of the liver's lipid composition in the context of the development of experimental insulin resistance (IR) induced by alimentary obesity and reduces the manifestations of steatosis. NSE was found to restore the structural and functional state of adipose tissue in rats with IR. These data have been published in several publications [26-33]. Given that the chronic low-gradient inflammatory process plays an increasingly important role in the pathogenesis of Type 2 diabetes mellitus, we investigated the anti-inflammatory effect of NSE in experimental IR in rats. Further studies using isolated peritoneal macrophages in rats with IR demonstrated that NSE inhibits the translo-

cation of the transcription factor NF-kB into the cell nucleus. Further, data were obtained that indicate the possibility of interaction of NSE with nuclear PPAR-gamma receptors, which leads to inhibition of translocation of transcription factor NF-kB into the nucleus of rat immune system cells with IR and a decrease in the production of inflammatory mediators (cytokines TNFα, Il1α/β). The possibility of such an interaction has been confirmed *in silico* and *in vitro* studies [34-36]. PPAR-gamma is recognized as a key regulator of glucose and lipid metabolism, insulin sensitivity and inflammation, promoting the anti-inflammatory activation of M2 macrophages (Fig. 1). Today, PPAR-gamma is assigned the role of the primary regulator of adipocyte differentiation. Therefore, PPAR-gamma may be the target of the interaction that determines the NSE effects identified by us in modeling experimental insulin resistance and other pathological conditions.

It is known that one of the complications of Type 2 diabetes mellitus is the development of neurodegenerative changes, manifested by loss of cognitive functions and memory.

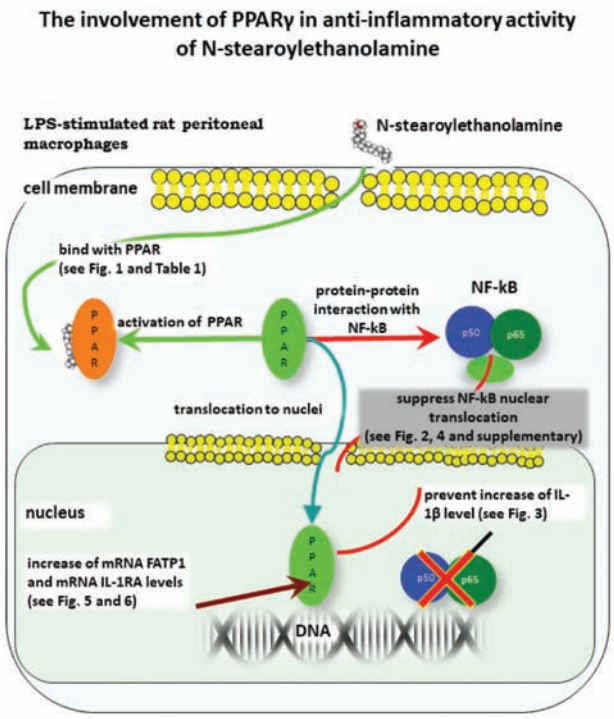


*Fig. 1. Illustration of PPARγ involvement in the anti-inflammatory action of NSE. Primarily, NSE inhibited nuclear translocation of NF-κB in LPS-stimulated peritoneal macrophages through PPARγ. In addition, NSE treatment altered hepatic mRNA expression of PPARγ target genes (Slc27a1, Il1rn) in insulin-resistant rats [36]*

In experimental models of cholinergic deficiency and social stress in rats, we established the anti-amnesic effect of NSE. It turned out that the use of NSE helps to reduce the activity of acetylcholinesterase and, thus, improves the functioning of the brain's cholinergic system. Additionally, NSE promotes the restoration of the lipid composition of the hippocampus and frontal cortex – structures of the mammalian brain responsible for the formation of cognitive functions and memory. Apparently, the resulting effects of NSE underlie its anti-amnesic action and restoration of cognitive function in rats. The research results are published in several scientific articles [37, 38], and medicines developed based on these studies are protected by patents [39, 40].

In general, the results of studies on the biological action of NSE in insulin resistance and cognitive impairment indicate that the action of NSE is systemic, aimed at restoring metabolic and structural changes in cells and organs caused by the development of pathological conditions.

Another biological process where acute and chronic inflammatory processes are very pronounced is aging. Therefore, our follow-up research from 2019 aimed to investigate the effect of exogenous NAE during aging complicated by the inflammatory process.

## Investigation of the mechanisms of geroprotective action of N stearoylethanolamine in normal and pathological aging

The need for such studies has resulted from the fact that aging is an irreversible biological process. However, there are numerous factors and chronic diseases that accelerate the aging process, such as cardiovascular diseases, cancer, diabetes, chronic stress, chronic inflammatory processes, dementia, neurodegeneration, etc., which worsen the quality of life of older people.

Aging is a complex of changes in the body that progressively and adversely lead to a general decrease in its biological functions, making it more susceptible to diseases and, ultimately, to death. Over time, the body's immune system function decreases, typically accompanied by a low-grade inflammatory condition. These conditions of local or generalized chronic inflammation (which is accompanied by typical phenomena of cellular aging, such

as telomere loss, oxidative stress, and DNA defects) damage all organs, which eventually leads to the development of age-related diseases such as osteoporosis, osteoarthritis, atherosclerosis, neurodegenerative disorders, and cancer. Recently, there has been more and more information in the literature that increased levels of pro-inflammatory cytokines, such as IL-1β, IL-6, TNFα interleukins, and defects in Innate and adaptive immunity are a likely key mechanism by which biological, chemical, and psychological chronic stressors can have powerful consequences for human health, accelerating the risk of age-related diseases. Moreover, growing evidence suggests that controlling the inflammatory state may provide a better chance of "healthy" aging.

As a result of the conducted studies, it turned out that NSE has a protective effect on the aging process in rats. Thus, the use of the drug led to the normalization of the state of the antioxidant system, the content of nitrite-anion and pro-inflammatory cytokines, positive modulation of the lipoprotein profile of blood plasma, normalization of the lipid and fatty acid composition of the heart and elimination of age-related morphofunctional changes in the myocardium [41]. Our studies have shown that administration of NSE to old rats has a corrective effect on aging-induced changes in brain lipidome; namely, it eliminates the imbalance of fatty acids, normalizes the content of free cholesterol and phospholipids, and contributes to a likely increase in the content of plasmalogenic forms of phosphatidylethanolamine in the hippocampus and frontal cortex of the brain of old rats.

Regarding cognitive impairment during aging, NSE in older rats was found to improve short-term memory, increase research activity, and reduce anxiety. As a result of our research, our hypothesis was experimentally confirmed that this effect of NSE is based on an increase in compensatory decrease in acetylcholinesterase activity in the brain and an increase in enzyme activity in blood plasma, as well as a decrease in choline content and an increase in acetylcholine content in the frontal cortex of the brain of old rats to values in young animals.

From our point of view, it would be exciting to study the effect of NAE on morphofunctional changes in the brain during the aging process. In studies conducted with the participation of colleagues from the laboratory of morphology and cytology of the D. F. Chebotarev Institute of Gerontology of the National Academy of Medical Sciences of Ukraine, it was found that the use of NSE also contributes to a decrease in the number of hyperchromic and vacuolized forms of neurons in the hippocampus and frontal cortex of old rats, which indicates a decrease in the manifestations of age-related metabolic changes. Thus, the action of NSE is aimed at reducing the manifestations of age-related brain changes in older animals.

Another confirmation of our hypothesis about the anti-inflammatory effect of NAE by the receptor mechanism was the data obtained in 2019. Thus, a single administration of NSE to old rats, both 60 min before and 60 min after the induction of acute inflammation in them, causes inhibition of translocation of NF-kB factor into the nucleus of peritoneal macrophages, which is accompanied by a decrease in the content of pro-inflammatory cytokines TNFα and IL-1β in the blood of animals and indicates a pronounced anti-inflammatory effect. NSE was found to help reduce anxiety, restore short-term memory, and research activity levels in older rats with induced chronic inflammation.

In 2020-2021, we found that aging is associated with a decrease in the content of nuclear PPAR-gamma receptors in rat blood serum, leading to an increase in circulating levels of pro-inflammatory cytokines (TNFα and IL-12) and the development of a chronic inflammatory process. The use of NSE in elderly rats *per os* at a dose of 50 mg/kg of body weight for 10 days results in an increase in the content of PPAR-gamma and a decrease in the content of these pro-inflammatory cytokines in the blood serum of the animals. It was found that during the development of the bacterial lipopolysaccharide-induced inflammatory process in old rats, the content of PPAR-gamma in the blood serum increases compensatory, compared with the indicators of its content in intact animals. However, the content of pro-inflammatory cytokines increases significantly, indicating a deficiency of endogenous PPAR-gamma agonists. When NSE per os was administered to elderly rats at a dose of 50 mg/kg of body weight for 10 days during the simulation of chronic inflammation in them, the levels of PPAR-gamma and pro-inflammatory cytokines in the blood serum of animals remained at the level of values characteristic of age control. These results indicate that there is no development of an induced inflammatory process in rats when using NSE in the process of modeling inflammation.

It was found that the protective effect of NSE on the content of polyunsaturated FA during normal

aging occurs due to the normalization of the content of ω6 FA, and during pathological aging – both ω6 (to a greater extent) and ω3 FA. It was found that the use of NSE per os at a dose of 50 mg/kg body weight for 10 days in elderly rats with induced chronic inflammation helps reduce anxiety, restore short-term memory, and increase the level of research activity to values characteristic of control animals. As a result of our studies, it was found that the content of corticosterone, epinephrine and dehydroepiandrosterone (DHEA) in blood plasma significantly decreases in old rats (18-month-old) compared with the values in young (4-month-old) rats. The oral administration of NSE to old rats at a dose of 50 mg/kg of body weight for 10 days increases the content of corticosterone and DHEA in blood plasma to levels found in young animals, but does not affect the level of epinephrine. It was found that the development of a chronic bacterial lipopolysaccharide-induced inflammatory process in old rats causes an even greater decrease in the content of corticosterone, epinephrine and DHEA in the blood plasma of animals. The use of NSE *per os* at a dose of 50 mg/kg of body weight for 10 days in old rats in the process of modeling chronic inflammation in them contributes to an increase in corticosterone and DHEA levels to values characteristic of age-matched controls and increases epinephrine content to values characteristic of young rats.

It was found that the content of adrenocorticotropic hormone (ACTH) in blood plasma was significantly higher in old rats compared to young rats. The use of NSE to elderly rats *per os* at a dose of 50 mg/kg of body weight for 10 days causes a significant decrease in ACTH content. An even greater increase in plasma ACTH was observed in the chronic bacterial lipopolysaccharide-induced inflammatory process in old rats. The use of NSE *per os* at a dose of 50 mg/kg of body weight for 10 days in elderly rats in the process of modeling chronic inflammation in them helps reduce ACTH levels to values characteristic of age-related controls. These results indicate a pronounced relationship between the hypothalamic-pituitary-adrenal and endocannabinoid systems in implementing the response to the development of chronic inflammation during aging. These data demonstrate the potential of NSE as an effective modulator of these regulatory systems of the body, both in normal and complicated aging with the development of chronic inflammation.

Thus, the results of the conducted studies demonstrate a pronounced geroprotective effect of NSE in both normal and pathological aging, which consists in increasing the antioxidant potential, inhibiting chronic low-grade inflammation, normalizing lipid metabolism, increasing adaptive potential, pronounced cardio- and neuroprotective effects, improving cognitive functions and memory status in old rats [42, 43]. The obtained results expand our understanding of the mechanisms of biological action of N-stearoylethanolamine and provide the basis for the development of a drug based on it, which can be used in complex therapy for the prevention and treatment of diseases associated with the aging process, thereby improving the quality of life of older people.

### Antiviral effect of NAEs

A separate area of work of the department is the study of antiviral properties of NAE. The impetus for testing the hypothesis that NAE can be a powerful antiviral substance was the well-known fact of using NPE as a preventive agent against respiratory infections under the commercial name “Impulsin” in former Czechoslovakia in the 1970s. At that time, this NAE was obtained from rotten chicken eggs, so it was not widely used in medical practice.

Studies of the antiviral properties of NAE were conducted in cooperation with employees of the laboratory of antiviral drugs of the Gromashevsky Institute of Virology of the National Academy of Medical Sciences of Ukraine (headed by the Doctor of Medical Sciences, Professor S. V. Rybalko). In vivo and *in vitro* experiments investigated the effect of NAE on the activity and replication of influenza, herpes simplex, hepatitis, and coronavirus viruses.

Already at the beginning of the research, the ability of saturated C18:0 NAE NSE in very small doses (0.0817 μg/kg body weight), which is several thousand times less than the effective dose of the well-known anti-influenza drug oseltamivir (commercial name “Tamiflu” , therapeutic dose 10 mg/kg body weight) to inhibit the development of the H1N1 influenza virus in mice was striking [44-46].

As a result of research, SANOFLU® with antineuraminidase and interferon-inducing effects was developed for the prevention and treatment of influenza infections.

The action of SANOFLU® is realized by inhibiting the neuraminidase activity of the influenza virus and inducing the production of gamma – and alpha-interferons in the infected body, protecting cells from atypical proliferative activity. The product

does not lose its antiviral activity for a long time after application (up to 5 days).

SANOFLU® is used by applying it in intranasal form. The index of anti-flu effectiveness of SANOFLU® is 100% for both therapeutic and preventive administration schemes (Fig. 2) [47].

Further studies have shown that NSE can also be considered as an effective treatment for human hepatitis C [49]. NSE inhibits the reproduction of surrogate hepatitis C virus (bovine diarrhea virus) at concentrations of $10^{-8}$ and $10^{-9}$ M relative to the control. The drug in concentrations from $10^{-6}$ M to $10^{-9}$ M with high efficiency (100%) suppresses the reproduction of the human hepatitis C virus in human cells transfected with the hepatitis C virus. The results of studies indicate a strong hepatoprotective and antiviral effect of the drug against the human hepatitis C virus, which indicates its suitability for use as a substance with combined antiviral and hepatoprotective effects in the treatment of the human hepatitis C [49].

In further studies of the antiviral effect of NSE, it was found that NAE also exhibits pronounced antiviral activity against the herpes simplex virus, both *in vitro* and *in vivo* studies, and is effective in both preventive and therapeutic regimens [50]. At the same time, the effective dose of the drug with the therapeutic administration scheme is 0.2 mg/kg, with the use of which 75% of animals survived. In contrast with the action of virolex at a dose of 10 mg/kg, – 50% of mice with herpetic meningoencephalitis survive. The mechanism of this antiherpetic action of NAE is to inhibit the reproduction of the herpes simplex virus. Thus, NSE effectively inhibits the reproduction of herpes virus Type 2 at concentrations from $10^{-6}$ to $10^{-8}$ in Vero cell culture (chemotherapeutic index = 103).

Studies on the anti-coronavirus activity of SANOFLU®, conducted in March-April 2020, at the very beginning of the SARS-CoV-2 virus pandemic, were very promising. On the *in vitro* model of coronavirus transmissible gastroenteritis in pigs, it was shown that in SNEV cells affected by coronavirus, when present in the SANOFLU® cultivation medium, a decrease in the infectious titer of the virus was observed in all studied concentrations ($10^{-3}$–$10^{-9}$ M).

At the same time, the lowest effective concentration ($EC_{50}$) of SANOFLU® is $10^{-9}$ M, and the selectivity index is 104, which indicates its high antiviral activity.

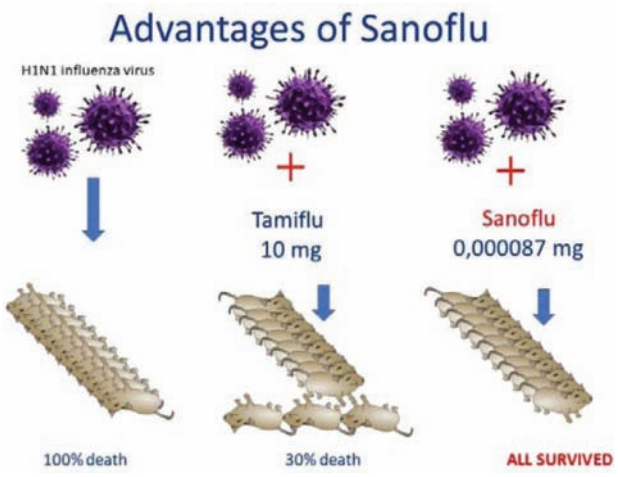


*Fig. 2. Illustration of the antiviral efficacy of the medications "Sanoflu" and "Tamiflu"*

Thus, SANOFLU® effectively reduces the replication of coronavirus and, due to its pharmacological properties, in particular, anti-inflammatory, antiplatelet and antioxidant, can be considered as a promising tool for the prevention and treatment of coronavirus disease [51].

Analyzing the obtained data, we hypothesized that the antiviral effect of NSE can be attributed to its interaction with specific viral proteins, thereby blocking the viral particle's ability to enter the cell or preventing it from replicating within the host cell. Research to test this hypothesis has already begun. As for the ability of SANOFLU® to inhibit hepatitis C virus replication, *in silico* studies have shown that this can occur due to The Binding of NSE to the active center of the viral protein protease NS3 and RNA-dependent RNA polymerase NS5B, which are responsible for viral replication [47].

*Conclusion*. In the Department of Lipid Biochemistry, a new class of low-polar minor lipids – N-acylethanolamines - was discovered, and a multi-faceted detailed study of their biological activity and functional role in the body was conducted. This made it possible to discover several new mechanisms for regulating vital processes, both in normal and various pathological conditions, and created prerequisites for the development of fundamentally new drugs. These products are offered for use in cardiovascular diseases, allergies, burns, type I and type II diabetes, inflammatory processes, cancer, organ transplantation, as well as drug addiction, alcoholism, viral infections, stress, etc. The products are protected by 19 Ukrainian patents.

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

## ІСТОРИЧНИЙ ОГЛЯД ДОСЛІДЖЕНЬ ВІДДІЛУ БІОХІМІЇ ЛІПІДІВ: ВІД ПЕРШИХ ГІПОТЕЗ ДО З'ЯСУВАННЯ БІОЛОГІЧНОЇ РОЛІ N-АЦИЛЕТАНОЛАМІНІВ

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У відділі біохімії ліпідів Інституту біохімії ім. О. В. Палладіна НАН України протягом 1988-2025 років було розроблено та запроваджено системний підхід щодо вивчення функціональної ролі ліпідів та дослідження ліпідому ссавців та людини. Було відкрито новий клас малополярних ліпідів – N-ацилетаноламінів (NAE) і проведено багатопланове детальне дослідження їх біологічної активності та функціональної ролі в організмі. Це дозволило відкрити низку нових механізмів регуляції життєво важливих процесів як в нормі, так і за різних патологічних станів. Актуальність цих досліджень полягає в тому, що вони дозволили не тільки поглибити фундаментальні знання в біології людини і тварин, але й привели до розробки низки фармакологічних засобів для терапії цілий ряду патологічних станів. Препарати пропонуються для використання при серцево-судинних захворюваннях, алергіях, опіках, цукровому діабеті I та II типу, запальних процесах, при онкологічних захворюваннях, трансплантації органів, а також за хронічного та гострого стресу, наркоманії та алкоголізмі та посттравматичному стресовому розладі. Також розроблено засоби, що мають потужну антивірусну дію відносно вірусу грипу, гепатиту С, вірусу простого герпесу та коронавірусу. Ці засоби не мають побічних ефектів і захищені 19 патентами України. Наукові результати відділу біохімії ліпідів опубліковано у понад 200 наукових працях, представлені на більш ніж 130 закордонних та вітчизняних наукових форумах. В цій статті коротко розглядаються основні досягнення відділу біохімії ліпідів у вивченні біологічної дії NAE.

Ключові слова: ліпіди, N-ацилетаноламіни, ендоканабіноїдна система, патологічні стани.

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