



ACTIVITY MODULATION OF VARIOUS NITRIC OXIDE SYNTASES AS AN APPROACH TO ENDOTHELIAL DYSFUNCTION THERAPY

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Received 16 Nov 2021

After peer review 10 Feb 2022

Accepted 11 March 2022

Nitric oxide as a therapeutic approach to the treatment of cardiovascular diseases attracted the attention of researchers at the end of the 19th century. As a vasodilator, nitric oxide may be a unique therapeutic agent for the treatment of hypertension and, as a result, renal failure and left ventricular hypertrophy.

The aim of the article is to analyze the literature data on possible ways of modulating the activity of various nitric oxide synthases as an approach to the treatment of endothelial dysfunction.

Materials and methods. When searching for materials for writing a review article, such abstract databases as PubMed, Google Scholar, e-Library, etc., were used. The search was carried out on the publications for the period from 1990 to 2021. The following words and phrases were chosen as parameters for the literature selection: nitric oxide; NO synthase; endothelial dysfunction; NO synthase activator; NO synthase inhibitor.

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Results. The article presents the history of the nitric oxide discovery and its biological role, the process of its biosynthesis, as well as the isoforms of its synthesizing enzymes (NOS): neuronal – nNOS, endothelial – eNOS and inducible iNOS, and their role in normal and pathological physiology. The process of NOS uncoupling (its molecular mechanisms) has been considered as the basis of endothelial dysfunction.

The examples of the pharmacological correction (BH_4 , arginase inhibitors, statins, resveratrol) are presented. In addition, NO synthase activators (calcium dobesilate, cavNOxin, and some NOS transcription activators), as well as non-selective (L-NMMA, 1-NNA, L-NAME, ADMA, 546C88, VAS203) and selective (L-NIO, 7-nitroindazole, aminoguanidine, L-NIL, GW273629, GW274150, cavtratin) inhibitors of nitric oxide synthase have been described.

Conclusion. Nitric oxide synthases continue to be promising targets for the development of agents that modulate their activity to correct various pathologies. As a therapeutic approach, modulation of the nitric oxide synthase activity can be implemented to treat endothelial dysfunction, which is the cause for complications of many diseases.

Keywords: nitric oxide (II); NO synthase; endothelial dysfunction; cavNOxin; resveratrol; aminoguanidine

Abbreviations: 7-NI – 7-Nitroindazole; ADDP – 6-Acetyl-7,7-dimethyl-5,6,7,8-tetrahydropterin; ADMA – asymmetric dimethylarginine; AMPK – adenosine monophosphate-activated protein kinase; AP-1 – activating protein-1; AP-2 – activating protein-2; ApoE – apolipoprotein E; BH_2 – dihydrobiopterin; BH_3 – trihydrobiopterin; BH_4 – tetrahydrobiopterin; CaD – Calcium Dobesilate; cNOS – constitutive nitric oxide synthase; CO – carbon monoxide; DDAH – dimethylarginine dimethylaminohydrolase; DHFR – dihydrofolate reductase; eNOS, NOS3 – endothelial nitric oxide synthase; ER – estrogen receptor; FGF – fibroblast growth factor; FPP – farnesyl pyrophosphate; GGPP – geranylgeranyl diphosphate; GSH – glutathione; GSSG – glutathione disulfide; GTPCH-I – guanosine triphosphate cyclohydrolase I; HUVEC – human umbilical vein endothelial cells; IFN- γ – interferon gamma; iNOS, NOS2 – inducible nitric oxide synthase; L-NAME – N^G -nitro-L-arginine; L-NIO – $\text{N}(5)$ -(1-Iminoethyl)-L-ornithine; L-NMMA – N^G -monomethyl-L-arginine; L-NNA – N^G -nitro-L-arginine; NF-1 – neurofibromin 1; NF-kB – nuclear factor-kB; nNOS, NOS1 – neuronal nitric oxide synthase; NO – nitric oxide (II); NOHA – N^G -hydroxy-L-arginine; NOCCL – NO-cleavable cross-linker; Nrf2, NFE2L2 – nuclear factor erythroid 2, (NF-E2)-related factor 2; ODQ – 1H-[1,2,4] oxadiazolo[4,3-a]quinoxalin-1-one; PCSK9 – proprotein convertase subtilisin/kexin type 9; PI3K – phosphoinositide 3-kinase; PKA – protein kinase A; PKC – protein kinase C; ROCK – Rho-associated protein kinase; ROS – reactive oxygen species; SREBP-2 – Sterol regulatory element-binding protein 2; TGF – transforming growth factor; TNF α – Tumor necrosis factor alpha; VEGF-A – vascular endothelial growth factor A; ABP – arterial blood pressure; ROI – reactive oxygen intermediate; GTP – Guanosine-5'-triphosphate; DNA – deoxyribonucleic acid; GIT – gastrointestinal tract; LDL(s) – low-density lipoproteins; LPS – lipopolysaccharide; mRNA – messenger ribonucleic acid; RA – rheumatoid arthritis; sGC – soluble guanylate cyclase; FAD – flavine adenine dinucleotide; FMN – flavin mononucleotide; cGMP – cyclic guanosine monophosphate; CNS – central nervous system.

For citation: D.V. Kurkin, E.E. Abrosimova, D.A. Bakulin, N.S. Kovalev, M.A. Dubrovina, A.V. Borisov, A.V. Strygin, E.I. Morkovin, I.N. Tyurenkov. Activity modulation of various nitric oxide synthases as an approach to endothelial dysfunction therapy. *Pharmacy & Pharmacology*. 2022;10(2): 130-153. DOI: 10.19163/2307-9266-2022-10-2-130-153

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Для цитирования: Д.В. Куркин, Е.Е. Абросимова, Д.А. Бакулин, Н.С. Ковалев, М.А. Дубровина, А.В. Борисов, А.В. Стрыгин, Е.И. Морковин, И.Н. Тюреньков. Модуляция активности различных синтаз оксида азота в качестве подхода к терапии эндотелиальной дисфункции. *Фармация и фармакология*. 2022;10(2):130-153. DOI: 10.19163/2307-9266-2022-10-2-130-153

МОДУЛЯЦИЯ АКТИВНОСТИ РАЗЛИЧНЫХ СИНТАЗ ОКСИДА АЗОТА В КАЧЕСТВЕ ПОДХОДА К ТЕРАПИИ ЭНДОТЕЛИАЛЬНОЙ ДИСФУНКЦИИ

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Получена 16.11.2021

После рецензирования 10.02.2022

Принята к печати 11.03.2022

Оксид азота как терапевтический подход к лечению сердечно-сосудистых заболеваний привлек внимание исследователей еще в конце XIX века. Являясь вазодилататором, оксид азота может быть уникальным терапевтическим средством для лечения гипертензии и, как следствие, почечной недостаточности и гипертрофии левого желудочка.

Цель. Проанализировать данные литературы о возможных путях модуляции активности различных синтаз оксида азота в качестве подхода к терапии эндотелиальной дисфункции.

Материалы и методы. При поиске материала для написания обзорной статьи использовали такие реферативные базы данных, как PubMed, Google Scholar, e-Library, и др. Поиск осуществлялся по публикациям за период с 1990 по 2021 гг. Параметрами для отбора литературы были выбраны следующие слова и словосочетания: оксид азота; NO-синтаза; эндотелиальная дисфункция; активатор NO-синтазы; ингибитор NO-синтазы.

Результаты. В статье представлена история открытия оксида азота и его биологическая роль, процесс его биосинтеза, а также изоформ ферментов его синтезирующих (NOS): нейрональной – nNOS, эндотелиальной – eNOS и индуцибельной iNOS и их роль в нормальной и патологической физиологии. Рассмотрен процесс разобщения NOS (его молекулярных механизмов) в качестве основы эндотелиальной дисфункции. Представлены примеры фармакологической коррекции (BH₄, ингибиторы аргиназы, статины, ресвератрол). Кроме того, описаны активаторы синтаз NO (кальция добезилат, cavNOxin, и некоторые активаторы транскрипции NOS), а также неселективные (L-NMMA, 1-NNA, L-NAME, ADMA, 546C88, VAS203) и селективные (L-NIO, 7-нитроиндазол, амингуанидин, L-NIL, GW273629, GW274150, кавтрапин) ингибиторы синтаз оксида азота.

Заключение. Синтазы оксида азота продолжают оставаться перспективными мишенями для разработки средств, модулирующих их активность для коррекции различных патологий. В качестве терапевтического подхода модуляция активности синтазы оксида азота может быть реализована для лечения эндотелиальной дисфункции, являющейся причиной осложнений многих заболеваний.

Ключевые слова: оксид азота (II); NO-синтаза; эндотелиальная дисфункция; cavNOxin; ресвератрол; амингуанидин

Список сокращений: 7-NI – 7-нитроиндазол; ADDP – 6-ацетил-7,7-диметил-5,6,7,8-тетрагидроптерин; ADMA – асимметричный диметиларгинин; AMPK – аденозинмонофосфат-активируемая протеинкиназа; AP-1 – activating protein-1 (активирующий белок-1); AP-2 – активирующий белок-2; ApoE – аполипопротеину E; BH₄ – дигидробиоптерин; BH₃ – тригидробиоптерин; BH₄ – тетрагидробиоптерин; CaD – кальция добезилат; cNOS – конститутивная изоформа NO синтазы; CO – монооксид углерода; DDAN – диметиларгинин диметиламиногидролаза; DHFR – дигидрофолатредуктаза; eNOS, NOS3 – эндотелиальная синтаза оксида азота; ER – рецепторы эстрогена; FGF – фактор роста фибробластов; FPP – фарнезилпирофосфат; GGPP – геранилгеранилпирофосфат; GSH – глутатион; GSSG – дисульфид глутатиона; GTPCH-I – гуанозин трифосфат циклогидролаза I; HUVEC – эндотелиальные клетки пупочной вены человека; IFN-γ – интерферон-γ; iNOS, NOS2 – индуцибельная синтаза оксида азота; L-NAME – N^ε-нитро-L-аргинин; L-NIO – N(5)-(1-иминоэтил)-L-орнитин; L-NMMA – N^ε-монометил-L-аргинин; L-NNA – N^ε-нитро-L-аргинин; NF-1 – нейтрофилин 1; NF-kB – ядерный фактор-kB; nNOS, NOS1 – нейрональная синтаза оксида азота; NO – оксид азота (II); NOHA – N^ε-гидрокси-L-аргинин; NOCCL – NO-расщепляемый сшивающий агент; Nrf2, NFE2L2 – ядерный фактор-2, подобный фактору эритроидного происхождения 2; ODQ – 1H-[1,2,4]оксадиазоло-[4,3-a]хиноксалин-1-он; PCSK9 – пропротеин конвертаза субтилизин кексин 9; PI3K – фосфатидилинозитол-3-киназа; PKA – протеинкиназа A; PKC – протеинкиназа C; ROCK – Rho-ассоциированная протеинкиназа; ROS – активные формы кислорода; SREBP-2 – белок, связывающий регуляторный элемент стерола 2; TGF – трансформирующий фактор роста; TNFα – фактор некроза опухоли альфа; VEGF-A – фактор роста эндотелия сосудов A; АД – артериальное давление; АФК – активные формы кислорода; ГТФ – гуанозинтрифосфат; ДНК – дезоксирибонуклеиновая кислота; ЖКТ – желудочно-кишечный тракт; ЛПНП – липопротеины низкой плотности; ЛПС – липополисахарид; мРНК – матричная рибонуклеиновая кислота; РА – ревматоидный артрит; рГЦ – растворимая гуанилатциклаза; ФАД – флавинадениндуклеотид; ФМН – флавиномононуклеотид; цГМФ – гуанозинмонофосфат; ЦНС – центральная нервная система.

INTRODUCTION

Nitric oxide (II) (NO) is characterized by a wide range of properties: from a toxic air pollutant to a pro-inflammatory mediator and regulator of the most important body functions (vasomotor, neurotransmitter).

NO, a colorless toxic gas, was first studied by a British chemist Joseph Priestley in 1772. A student of Theophile-Jules Pelouze (who had developed nitrocellulose and other nitrosulfates), the Italian chemist Ascanio Sobrero, discovered nitroglycerin in 1847, for which he notified its

ability to reproducibly cause severe headaches. Another student of Peluz, Alfred Nobel, combined this highly unstable compound with kieselgur and patented it in 1867 as a more stable commercial explosive dynamite.

In the workers involved in the production of nitroglycerin, the disappearance of angina pectoris symptoms was notified. In 1879, the British physician William Murrell proposed nitroglycerin for the angina pectoris treatment. In 1977, Ferid Murad discovered that the positive effect of nitroglycerin on vascular smooth muscles is due to the release of NO. The 1998 Nobel Prize in Physiology and Medicine was shared by Ferid Murad and Robert F. Furchgott, who, together with John Zawadzki in 1980, established the importance of the endothelial relaxing factor caused by the vasodilating action of acetylcholine.

In the late 1970s, Robert Furchgott began to investigate vasoactive effects of acetylcholine, leading to the discovery of NO.

In the early 1980s, several studies were carried out, as a result of which the chemical properties and the action mechanisms of the endothelium-dependent relaxation factor were established. The similarity between the properties of NO and the properties of the endothelium-dependent relaxation factor had been determined by 1986.

In 1987, two separate laboratories – R. Furchgott and J. Zawadzki's, as well as Louis J. Ignarro and Salvador Moncada's [1] – published the definitive evidence that NO is an endothelium-dependent relaxation factor [2].

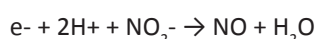
The discovery of NO as an endogenously generated signaling agent of a particular importance for the cardiovascular system set off a new era in the study of biological signaling in the late 1980s and early 1990s. This discovery changed the paradigm and demonstrated that a small, freely diffusible molecule can be synthesized endogenously to act as a specific signaling factor through interactions with a receptor protein [3].

Main effects of NO

NO plays an important role in the regulation of various physiological processes that are critical for functioning of many body tissues. When NO signaling is impaired, these central tissue processes can become pathophysiological, causing acute or chronic diseases in the CNS [4–7], blood vessels [8–10], gastrointestinal tract [11, 12], skeletal muscles [13, 14] and the immune system. [15, 16].

NO biosynthesis and signal transduction in the NO-ergic system

Endogenous NO is mainly generated by enzymatic pathways, but there is also a non-enzymatic pathway (Fig. 1A). A non-enzymatic NO production involves the production of NO from nitrite in several ways, especially under conditions of acidosis (e. g, after ischemia), and occurs primarily in tissues predominantly via a nitrite reduction:



THE AIM under conditions of ischemia with acidosis, anitrite-mediated NO production approaches a maximum constitutive nitric oxide synthase (NOS) production, making this pathway an alternative in ischemia, i.e. under the conditions when the NO production from NOS is violated [17].

MATERIALS AND METHODS

When searching for materials for writing a review article, such abstract databases as PubMed, Google Scholar, e-Library, etc. were used. The search was carried out on the publications for the period from 1990 to 2021. The following words and phrases were chosen as parameters for the literature selection: nitric oxide; NO synthase; endothelial dysfunction; NO synthase activator; NO synthase inhibitor.

RESULTS AND DISCUSSION

NO synthases and their role

The enzymatic production of NO is catalyzed by various NOS isoforms through a series of redox reactions in the presence of oxygen to cleave L-arginine to L-citrulline and NO via the intermediate metabolite N-hydroxy-L-arginine (NOHA), using NADPH as an electron donor.

Isolated NOS was first isolated by Bredt and Snyder in 1990 [18]. The three major mammalian NOS enzymes had already been cloned and successfully expressed in *E. coli* or insect cells and had been characterized by 1998.

Three major NOS isoforms have been identified based on their different primary amino acid sequences (only 50–60% identity), tissue and cellular distribution, and a regulation mechanism. Two NOS isoforms are calcium/calmodulin-dependent and constitutively expressed (cNOS, constitutive nitric oxide synthase): NOS of the endothelial origin (NOS3, eNOS, endothelial nitric oxide synthase) and NOS of the neuronal origin (NOS1, nNOS, neuronal nitric oxide synthase), respectively. The third NOS isoform is induced by cytokines, and its activity is independent on calcium/calmodulin (NOS2, iNOS, inducible nitric oxide synthase). The individual properties of each NOS isoform are of great importance, since they are: the magnitude, duration, and cellular sites of the NO production that determine the overall physiological or pathophysiological effect [19].

While iNOS normally produces large amounts of NO and is involved in the immune defense, inflammatory responses, and the NO production by airway epithelial cells, the constitutively expressed nNOS and eNOS isoforms typically generate nanomolar NO concentrations that are important for physiological processes such as neuronal signaling, inhibition of the hemostatic system, vasodilation and control of blood pressure (BP). NO synthesized in such quantities is not rapidly oxidized either,

and can directly interact with metal ions in proteins, for example, with the iron atom in the heme group of hemoglobin, myoglobin, soluble guanylate cyclase (sGC), cytochrome P450, and NOS itself [20].

All NOS isoforms use tetrahydrobiopterin (BH_4 , tetrahydrobiopterin), which is synthesized from guanosine triphosphate (GTP) by the enzyme GTP-cyclohydrolase-I (GTPCH-I). Other cofactors are flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and heme. All NOS proteins are homodimers.

Functional NOS transfers electrons from NADPH from the carboxy-terminal reductase domain through the flavins FAD and FMN to heme in the N-terminal oxygenase domain. The oxygenase domain also binds the important cofactor BH_4 , molecular oxygen, and the substrate L-arginine. At the heme site, electrons are used to reduce and activate O_2 , as well as to oxidize L-arginine to L-citrulline, followed by the release of NO.

To synthesize NO, the NOS enzyme goes through two stages. At the first stage, NOS hydroxylates L-arginine to N ω -hydroxy-L-arginine, which remains bound to the enzyme. At the second stage, NOS oxidizes N ω -hydroxy-L-arginine to L-citrulline and NO.

All the NOS isoforms bind calmodulin. In nNOS and eNOS, calmodulin binding is induced by an increase in intracellular Ca^{2+} concentration. When calmodulin's affinity for NOS increases, it facilitates the flow of electrons from NADPH in the reductase domain to heme in the oxygenase domain. In inducible NOS (iNOS), calmodulin binds at already extremely low intracellular Ca^{2+} concentrations due to the different amino acid structure of the calmodulin binding site (Fig. 1 B and C). All NOS proteins contain a zinc-thiolate cluster formed by a zinc ion that is tetrahedrally coordinated to two CysXXXXCys motifs (by one of each monomer) at the boundary of the NOS dimer. Zinc in NOS performs a structural rather than catalytic function [21].

Endothelial NO synthase

An endogenous NO production, especially in the cardiovascular system, mainly depends on the activity of the enzyme endothelial NO synthase. The eNOS enzyme was originally identified and isolated from bovine aortic endothelial cells [22, 23]. Further studies showed that several types of non-endothelial cells, such as cardiomyocytes [24], platelets [25], and neurons [26], also express eNOS.

In humans, the eNOS gene is located in the region 7q35–7q36 of chromosome 7 [27]. This gene is present as a single copy in the haploid set of the human genome.

Although eNOS is expressed constitutively, many factors regulate eNOS at transcriptional, post-transcriptional, and post-translational levels. In this regard, varia-

tions in the eNOS gene affect the regulation of the eNOS enzyme and, accordingly, the NO production [28].

Under physiological conditions, eNOS is responsible for the production of most endothelial NO, and for this reason, it plays a key role in the regulation of the cardiovascular system. This fact is constantly confirmed by clinical and experimental studies. In particular, eNOS knockout gene mice rapidly develop hypertension and have a high risk of stroke or other cardiovascular disease compared to wild-type mice. Similar disorders are also reproduced by a pharmacological inhibition of eNOS.

The regulation mechanisms of the eNOS activity are extremely complex and can be divided into two levels: genetic and protein. At the genetic level, the expression and stability of eNOS genes are associated with its activity. The eNOS promoter contains a large number of transcription factor binding sites, including activator protein-1 (AP-1), activator protein-2 (AP-2), the endothelin family, the nuclear factor- κ B (NF- κ B, nuclear factor- κ B), and neurofibromin 1 (NF-1). These transcription factor complexes can regulate the eNOS expression; and hypoxia, lipopolysaccharide, and several cytokines can induce it.

In quiescent endothelial cells, most of the expressed eNOS attaches to caveolae, pocket-like membrane invaginations rich in cholesterol and sphingolipids. The localization of eNOS in endothelial cell caveolae inactivates the enzyme as a result of the strong and direct interaction of eNOS with caveolin-1. This protein-protein interaction inhibits the eNOS activity mainly by interfering with the calmodulin binding site. In contrast, binding of calcium-activated calmodulin to eNOS displaces caveolin-1 and promotes the eNOS activation. Calmodulin interacts with a cognate binding site on eNOS located between the oxygenase and reductase domains. This binding shifts the adjacent autoinhibitory loop and allows NADPH-dependent electron flow to the enzyme heme. However, in the absence of bound calmodulin, the electron transfer is blocked and the eNOS catalytic activity is suppressed. In addition to caveolin-1, eNOS can interact with heat shock protein 90 (Hsp90), the protein involved in a multicomponent chaperone system that is responsible for the folding of several proteins. Hsp90 is involved in eNOS folding and can allosterically modulate the enzyme by inducing conformational changes or by stabilizing the dimeric form, thereby activating eNOS. It should be notified that the eNOS activity depends on the presence of a substrate (L-arginine) and cofactors (NADPH and BH_4) [28, 29].

In addition to these mechanisms, eNOS can be also activated by stimuli that do not cause a sustained increase in intracellular Ca^{2+} but still result in the sustained NO release. The main stimulus is a shear stress eNOS

can be phosphorylated by several serine (Ser), threonine (Thr), and tyrosine (Tyr) residues. Phosphorylation of Ser1177 stimulates the flow of electrons in the reductase domain, increases the sensitivity of the enzyme to Ca^{2+} , and represents an additional and independent mechanism of the eNOS activation [21].

Neuronal NO synthase

This type of NOS is expressed constitutively. The nNOS gene, located on chromosome 12, has a complex genomic organization. A cellular and tissue-specific regulation of nNOS is considered to be related to various transcription factors such as activator protein-2 (AP-2), transcription enhancer factor-1 (TEF-1)/MCAT binding factor, cAMP response element binding protein (CREB)/activating transcription factor/c-Fos, nuclear respiratory factor-1, Ets, nuclear factor-1 and NF-kappa B [30].

In addition, nNOS undergoes more than ten different alternative splicing options that potentially have unique structural features and catalytic properties. In the brain, the nNOS α form containing exon 2 is the main splicing variant, while nNOS μ is the predominant splicing variant in the skeletal muscle and the heart. nNOS-2, found in the mouse brain, acts as a dominant negative regulator of the nNOS activity due to the deletion in frame 105 of amino acid residues 504-608 involved in arginine binding. Both nNOS α and nNOS μ are attached to subcellular structures via the PDZ domain, while the other two forms, nNOS β and nNOS γ , are cytoplasmic [31].

Post-translational modifications represent an additional level of the nNOS regulation activity and include phosphorylation, ubiquitination, and interactions with other structural and regulatory proteins, resulting in the formation of a functional complex.

The enzymatic activity of nNOS is modulated by phosphorylation at multiple sites and promotes the interaction between NO and other signaling pathways through various protein kinases. Phosphorylation of nNOS can occur at a variety of residues, including serine (Ser), threonine (The), and tyrosine (Tyr). This fact is associated with the activation/inactivation of the enzyme directly, or with the modulation of other regulatory domains present in nNOS.

In addition to phosphorylation, ubiquitination and sumoylation are two major post-translational regulatory mechanisms involved in levels and functions controlling of several neuronal proteins. Protein ubiquitination is a signal for the degradation of dysfunctional proteins.

In brain cells, nNOS is present in bound and soluble forms. The differential subcellular localization of nNOS explains the diversity of its functions [21, 32].

The regulation mechanisms of nNOS are similar to those of eNOS: the calcium-binding protein, calmodulin,

acts as an allosteric activator and the nNOS activity is also regulated by heat shock proteins. Heat shock proteins (HSPs) comprise a family of molecular chaperones responsible for the proper folding and maturation of proteins. The Hsp90/Hsp70-based chaperone apparatus regulates the nNOS activity and turnover by modulating ligand-binding clefts [32].

The NOS of neurons contains a PDZ domain and can directly interact with similar domains of other proteins. These interactions determine the subcellular distribution and activity of the enzyme. The proteins containing the PDZ domain, such as syntrophin, PSD-95, or PSD-93, play an important role in the formation of multiprotein signaling complexes and are required for nNOS binding to various calcium sources. In skeletal muscles, nNOS is attached to the sarcolemma via syntrophin and dystrophin proteins. While in the CNS, nNOS connects to the cytoplasmic tail of NMDA-NR 2 via PSD95, which is responsible for calcium influx and the subsequent activation of nNOS. The subcellular fractionation of the brain tissue shows that about half of the nNOS in the brain is soluble, and the rest, via PSD95, is bound to the membrane fraction. PSD proteins are preferentially expressed at higher synaptic densities and interact with nNOS via PDZ domains [32, 33].

Neuronal NOS is involved in the modulation of physiological functions such as learning, memory, and neurogenesis. In the central nervous system, nNOS mediates the long-term regulation of synaptic transmission. NO formed in the CNS (under the action of nNOS) is involved in the central regulation of blood pressure [7]. In addition to the brain tissue, nNOS has been found in the spinal cord, sympathetic ganglia and adrenal glands, peripheral nerves, epithelial cells of various organs, nephrons, pancreatic islet cells, and vascular smooth muscle cells.

Inducible NO synthase

The iNOS gene is located on the 17th chromosome. The inducible isoform of NOS is mainly regulated at the level of expression. The mechanisms regulating the iNOS expression include the modulation of the promoter activity, the mRNA stability and translation, and finally protein. As with other enzymes, iNOS activity depends on the availability of the substrate. The stimuli and conditions that determine the iNOS expression depend on the functional state of the cell and its type. The inducers of the iNOS expression in one cell type may have no effect or even suppress an iNOS expression in another, even within the same organism [34].

Inducible NOS is not permanently expressed in cells, but its synthesis can be stimulated by bacterial lipopolysaccharide, pro-inflammatory cytokines (interferon-gamma, IL-1, IL-2, a tumor necrosis factor) or other agents,

such as reactive oxygen species, as well as hormones that affect the synthesis of cyclic adenosine monophosphate (adrenaline, glucagon). Although iNOS is primarily identified in macrophages, the expression of the enzyme can be stimulated in practically any cell or tissue, provided the appropriate inducing agents have been identified. After the expression, iNOS is constantly active and is not regulated by intracellular Ca^{2+} concentrations [35].

Inducible in macrophages, NOS produces a large amount of NO, which is the basis of the cytotoxicity of these cells. Due to its affinity for protein-bound iron, NO is able to inhibit the key enzymes the catalytic centers of which contain it. These include iron-sulfur cluster-dependent enzymes (complexes I and II) involved in the mitochondrial electron transport, ribonucleotide reductase (an enzyme that limits the rate of DNA replication) and cisconitase (a key enzyme in the citric acid cycle). Higher concentrations of NO produced by induced macrophages can directly damage the DNA of the target cells and cause chains breaks and their fragmentation. The combination of these effects probably underlies the cytostatic and cytotoxic effects of NO on parasitic microorganisms and some tumor cells. Interestingly, non-immune cells can be also induced by cytokines to release enough NO to affect neighboring cells. For example, cytokine-activated endothelial cells have been shown to lyse tumor cells, while induced hepatocytes can use NO to kill malaria sporozoites. The iNOS activity is probably responsible for all these effects [21].

Unlike eNOS and nNOS, iNOS has a much higher affinity for calmodulin, so it can bind it at very low Ca^{2+} concentrations and thus its activity is not regulated by Ca^{2+} /calmodulin, but mainly at the transcriptional level. The kinetic parameters of iNOS determine the catalytic activity level, which generates a higher amount of NO than eNOS and nNOS, and is less sensitive to the NO-dependent autoinhibition which contributes to the interaction of large amounts of NO with reactive oxygen species (ROS) and the generation of more toxic nitrogen species, such as nitrate [36].

The activity of expressed iNOS is regulated by various factors, including the availability of its substrate and cofactors, the interaction with other proteins, and the autoinactivation (to a lesser extent).

Several proteins interact with iNOS and regulate its activity. In the central nervous system (brain) of lipopolysaccharide (LPS) injected mice, iNOS is found complexed with a cytosolic protein called kalirin (primarily kalirin-7). Kalirin interacts with iNOS monomers, but not with iNOS dimers. In the cells expressing kalirin, most of the iNOS exists as a heterodimer with kalirin.

There is little monomeric iNOS, indicating that kalirin inhibits the iNOS activity, preventing its homodi-

merization. A low expression of kalirin correlates with the increased iNOS activity in the hippocampus of patients with Alzheimer's disease. As the excess NO produced during inflammation is neurotoxic, kalirin may have a neuroprotective effect by inhibiting iNOS. In macrophages, iNOS interacts with 110 kDa protein called NOS-associated protein 110 (NAP110). NAP110 and iNOS complexes are found in the spleen and peritoneal macrophages of interferon- γ (IFN- γ) and LPS-treated mice. A co-expression of NAP110 and iNOS reduces the activity of the latter by 90%, although the amount of the iNOS protein does not change. Like kalirin, NAP110 inhibits the iNOS activity by interacting with iNOS monomers and preventing enzyme dimerization. Therefore, NAP110 may be a blocking regulator that prevents the increase in NO levels from toxicity [37].

NO can negatively regulate the iNOS activity. In stimulated mouse macrophages, the iNOS activity is increased by the addition of hemoglobin, an NO acceptor. The addition of NO donors S-nitrosoacetylpenicillamine or S-nitrosoglutathione significantly inhibits the iNOS activity, suggesting that NO may be involved in a negative feedback mechanism, but much weaker than that of other isoforms. In addition, the iNOS activity cannot be restored in activated cells without NO donors, indicating irreversible binding of NO to iNOS. A kinetic study showed that the main mechanism of iNOS autoinactivation is S-nitrosylation of the zinc tetrathiolate cluster, which causes a zinc loss, an irreversible dissociation of the iNOS dimer and a subsequent loss of its activity. Reducing agents may protect iNOS from this inactivation, suggesting that S-nitrosylation was indeed the main inactivation pathway [37].

Mitochondrial NO synthase

Although NO is a highly diffusible molecule that can cross membranes and reach mitochondria, there is also evidence that mitochondria produce NO [38] and that mitochondrial NOS exists. However, the identity of this mitochondrial NOS is still under debate [39, 40]. Several studies have shown that various tissues such as skeletal muscles [14], the heart [42], kidneys, the brain [42] and liver [41, 42], contain NOS (possibly classified as mitochondrial) with a positive immunoreactivity for antibodies against iNOS [41], eNOS and nNOS. In addition, Gao S. et. al. [43] were able to better determine the localization of eNOS on the cytoplasmic surface of the outer mitochondrial membrane in endothelial cells [43]. Elfering S.L. et. al. characterized the rat mitochondrial NOS sequence as a nNOS α isoform that can be modified by acylation, it is calcium sensitive, and has phosphorylation sites [44]. They hypothesized that these post-translational modifications might be responsible for targeting NOS at the mitochondrial inner membrane.

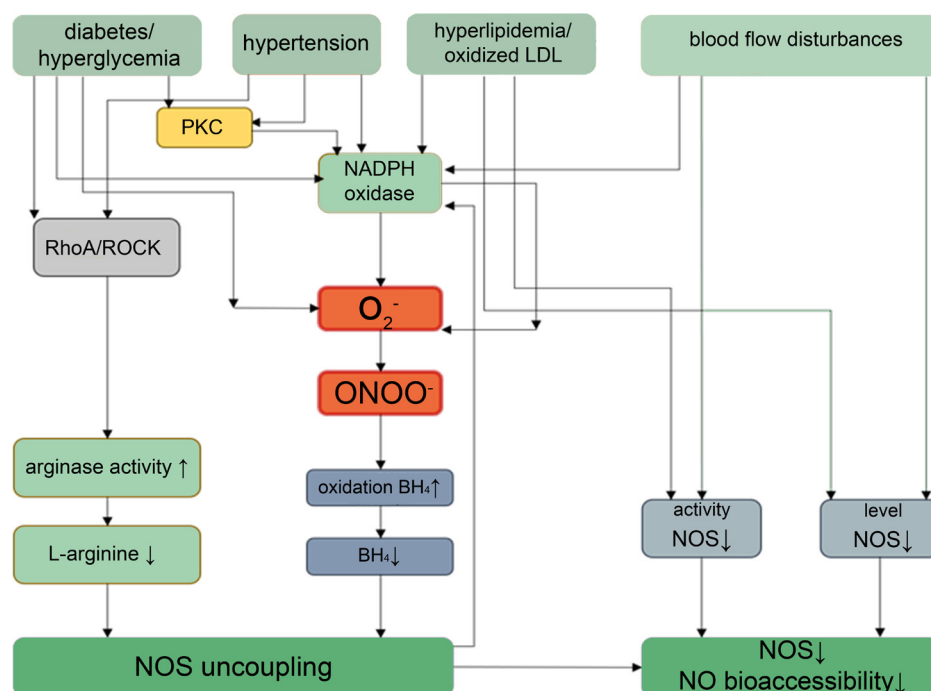


Figure 3 – Pathological conditions and mechanisms of NOS uncoupling

Note. Hypercholesterolemia, hypertension, smoking, and diabetes mellitus lead to the activation of NADPH (a reduced form of nicotinamide adenine dinucleotide phosphate) oxidase, partially by a protein kinase C (PKC)-dependent mechanism. Diabetes mellitus also stimulates the production of mitochondrial ROS, that then triggers the activation of NADPH oxidase, which can increase a mitochondrial production of superoxide (O_2^-) and xanthine oxidase. The endothelial NO synthase (eNOS) enzyme can be uncoupled through two main mechanisms: deficiency of the BH_4 cofactor or the L-arginine substrate. O_2^- reacts with NO to form peroxynitrite ($ONOO^-$). $ONOO^-$ oxidizes BH_4 resulting in BH_2 deficiency. L-arginine deficiency is caused by an increase in the arginase expression and activity, partially through RhoA/ROCK-dependent mechanisms. Unbound eNOS produces superoxide, thereby increasing the oxidative stress. The eNOS uncoupling reduces the endothelial NO production, which is further exacerbated by a decrease in the eNOS expression and activity. In addition to the population-level risk factors, the impaired blood flow makes arterial bifurcations and lateral branches prone to atherosclerosis. The increased oxidative stress and reduced endothelial NO production contribute significantly to the increased atherosclerosis in these areas. Adapted from [58].

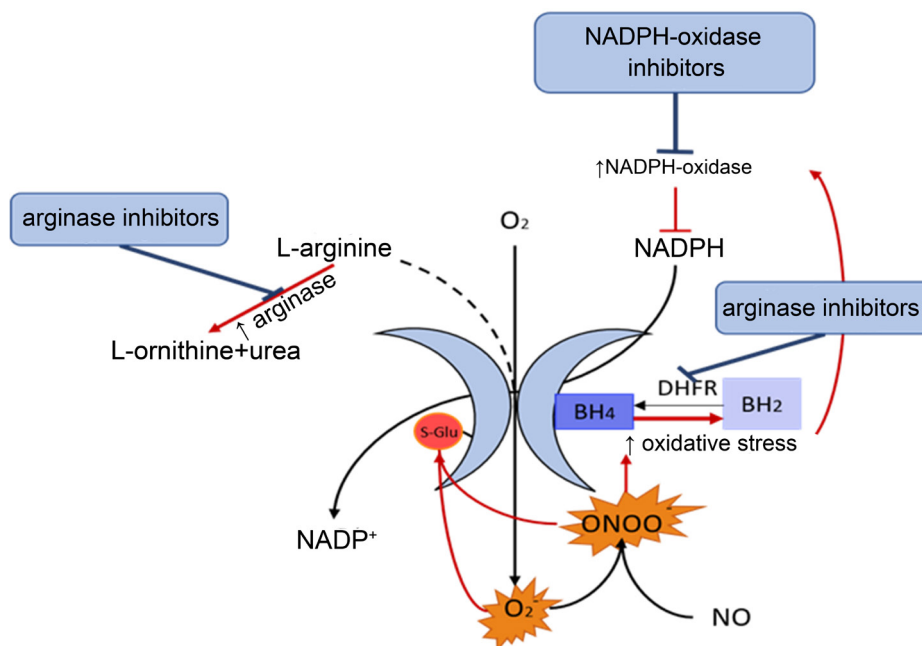


Figure 4 – Schematic representation of known causes and methods for correcting NOS uncoupling in the cardiovascular system

Note. Causes for NOS uncoupling are shown in red and treatments are shown in blue. Superoxide (O_2^-) or peroxynitrite ($ONOO^-$) can oxidize BH_4 to BH_2 , which can be prevented by antioxidants or NADPH oxidase inhibitors, and interfere with s-glutathionylation (s-Glu). Folic acid can stimulate dihydrofolate reductase (DHFR) to reduce BH_2 to BH_4 . Arginase inhibition can preserve L-arginine levels to make it available to the NOS enzyme. Adapted from [59].

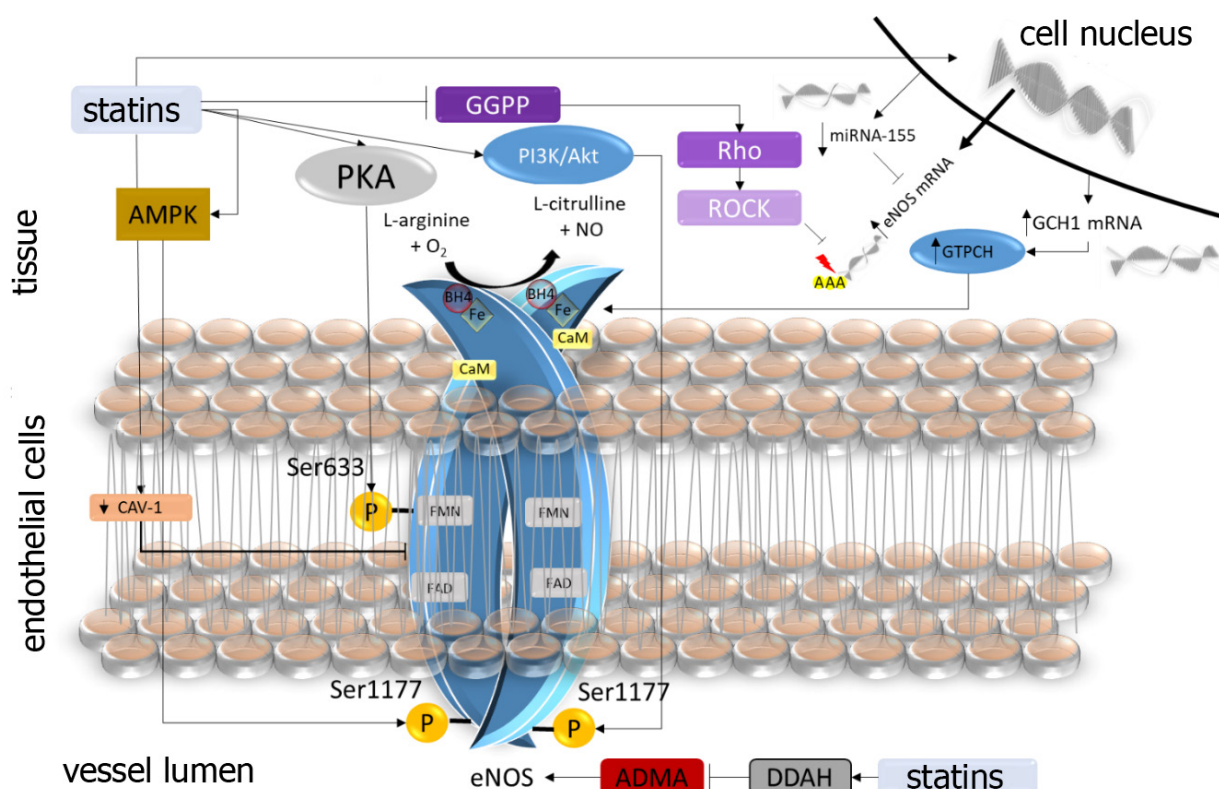


Figure 5 – Effect of statins on eNOS in endothelial cells

Note. By inhibiting mevalonate synthesis, statins reduce GGPP levels, prevent the Rho/ROCK activation and stabilize eNOS mRNA, thereby increasing the eNOS expression. By reducing caveolin-1 and circulating ADMA, as well as increasing eNOS phosphorylation at the Ser-633 and Ser-1177 activation sites via AMPK, Akt, and PKA, statins also increase the eNOS activity. Finally, by upregulating GTPCH, statins increase endothelial BH₄, the presence and restoration of eNOS binding. GGPP is geranylgeranyl pyrophosphate; ROCK – Rho-associated protein kinase; GTPCH – guanosine triphosphate cyclohydrolase; AMPK 2 adenosine monophosphate activated protein kinase; PKA, protein kinase A; PI3K – phosphatidylinositol-3-kinase; ADMA - asymmetric dimethylarginine. Adapted from [77].

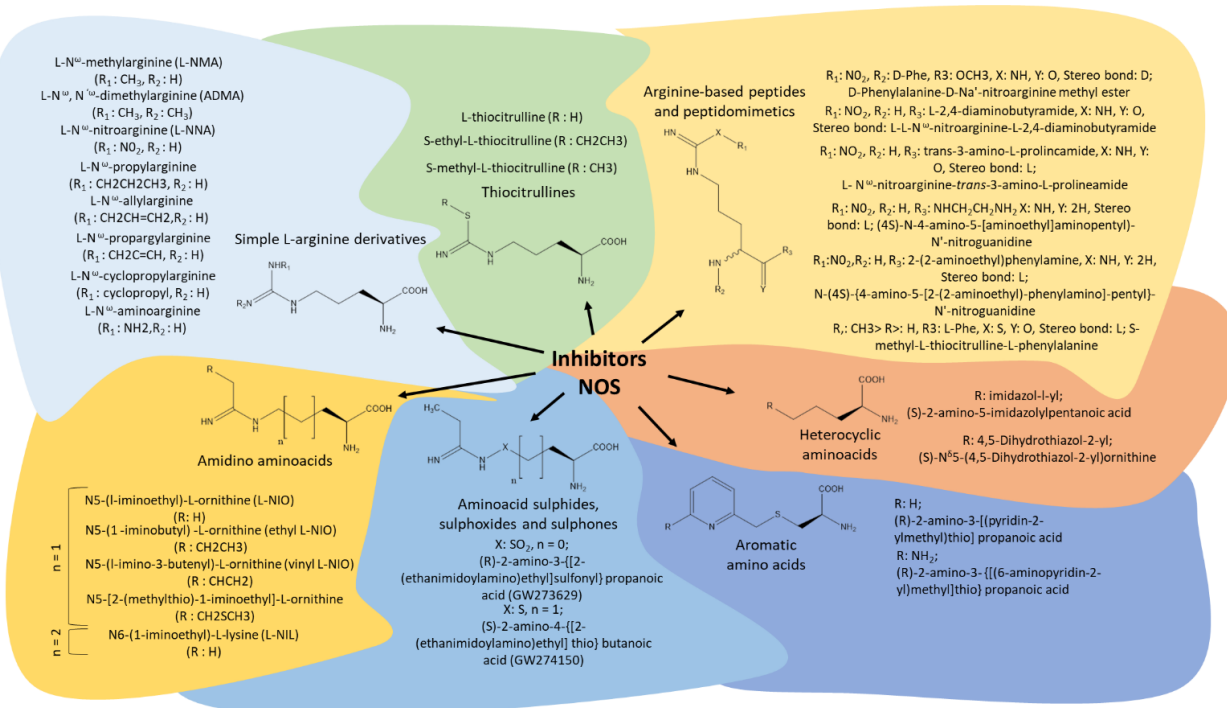


Figure 6 – List of the most important inhibitors based on arginine

Note. Adapted from [92].

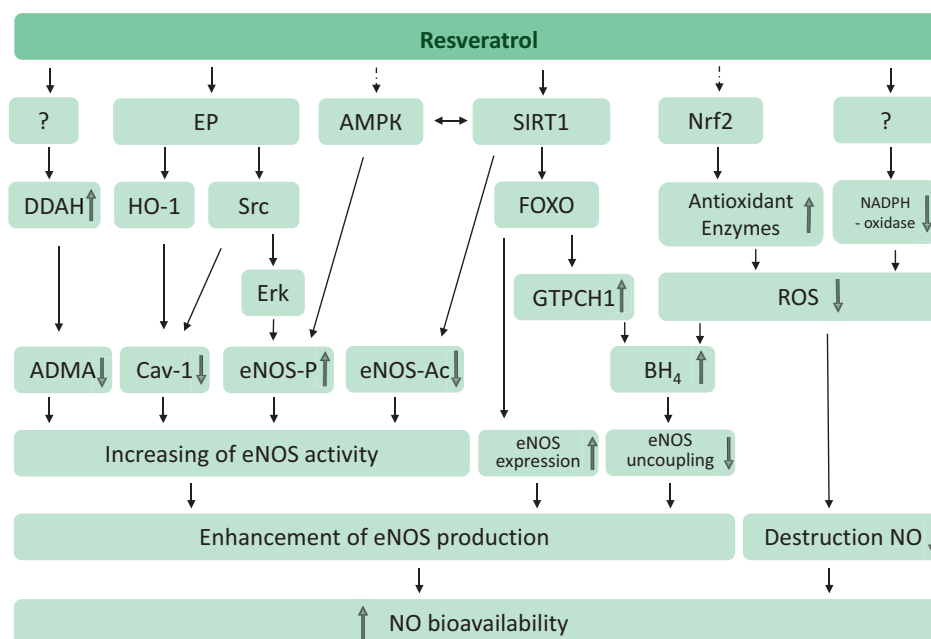


Figure 7 – Resveratrol targets to prevent NOS uncoupling (80 adapted)

Note. Resveratrol increases the NO production and prevents its breakdown. Resveratrol can activate sirtuin 1 (SIRT1) either directly (in a substrate-dependent manner) or indirectly (by inhibiting phosphodiesterases). SIRT1 stimulates endothelial NOS via deacetylation, enhances the eNOS expression via deacetylation of the forkhead box protein O1 (FOXO) transcription factor, and prevents the eNOS uncoupling by increasing the activity of GTP-cyclohydrolase I (GTPCH-I), a limiting factor in BH₄ biosynthesis. AMPK and nuclear factor 2 associated with Nrf2 are indirect targets of resveratrol. AMPK phosphorylates eNOS by serine 1177. eNOS can also be phosphorylated by Erk1/2, which is stimulated by a pathway involving the estrogen receptor (ER) and the Src family tyrosine kinase. Caveolin-1 (Cav-1) is a protein that negatively regulates the eNOS activity. ADMA is an endogenous eNOS inhibitor that is cleaved by DDAH. The targets for the activation of dimethylarginine dimethylaminohydrolase (DDAH) or inhibition of NADPH oxidase have not been identified yet. Adapted from [80].

Table 1 – Characteristics of various NO-synthase isoforms

Names	NOS-1 nNOS Neutral NOS NOSI	NOS-2 iNOS Induced NOS NOSII	NOS-3 eNOS Endothelial NOS NOSIII
Monomer size (person)	~160 kDa	~131 kDa	~133 kDa
Cofactors	NADPH; FMN; FAD; BH ₄ ; Calmodulin; heme		
Inhibitors	N-methyl-L-arginine N-nitro-L-arginine 7-nitroindazole 1-(2-trifluoromethylphenyl)imidazole L-thiocitrulline S-methyl-L-thiocitrulline	Aminoguanidine S-benzylisothiourea 1-(2-trifluoromethylphenyl)imidazole L-N6-(1-iminoethyl)lysine 1400W	N-methyl-L-arginine N-nitro-L-arginine N-iminoethyl-L-ornithine 7-nitroindazole
Intracellular localization	Cytosol; Plasma membrane [99]; Mitochondria [100]; Caveoli [101].	Plasma membrane [102]; Phagosomes [103]; Golgi complex; Mitochondria.	Plasma membrane; Lipid rafts; Caveoli [104]; Cytosol [100].
Cells	Neurons [105]; Islets of the pancreas [106]; Endothelial cells; Smooth muscle cells [107].	Macrophages and other leukocytes [108]; Smooth muscle cells; Cardiomyocytes [35].	Endothelial cells [109]; Platelets [25].
Tissue expression	Central and peripheral nervous system [105]; Skeletal muscles [110]; Endometrium [111]; Macula densa [112].	Heart; Liver [113]; Smooth muscles; Endothelium [113].	Endothelium [114].
Functions	Neurotransmission [105]; Renal tubular glomerular interactions [115]; Intestinal peristalsis [116].	Bactericidal activity; Cytotoxicity; Inflammation [117]; Septic shock [118].	Vascular relaxation Decreased platelet adhesion [119, 120]; Angiogenesis [121].
Involvement in diseases	Renal diseases [115]; Affective disorders [122].	Inflammation	Hypertension; Endothelial dysfunction.

Confirming the results obtained by Elfering S.L. et al., Kanai A.J. et al. showed that mitochondria isolated from the hearts of mice in which the nNOS α isoform had been knocked out, did not produce NO, while eNOS $-/-$ and iNOS $-/-$ knocked out the mice, retaining the NO production in isolated mitochondria [45]. Lacza Z. et al. were able to detect the NO production, the NOS activity, and low levels of eNOS protein in mitochondria. However, the NOS activity was not impaired in the eNOS knockout mice [46]. In addition, the mitochondrial NO production was not affected by the nNOS and iNOS knockout mice.

Venkatakrishnan P. et al. were unable to detect the activity or expression of endothelial and neuronal NOS isoforms in rat liver mitochondria, casting doubt on the existence of mitochondrial NOS, at least in the liver [47].

Regardless of this controversy over the existence or identity of mitochondrial NOS, the recent studies show a stronger relationship between mitochondrial NOS and the respiratory chain. Parihar et al. suggested that respiratory chain complex I regulates the mitochondrial NOS activity, demonstrating the complex I-associated mitochondrial NOS activity in the isolated rat liver and brain mitochondria [48]. In addition, a study of the cardiac mitochondrial preparations demonstrated that complex I enriched fractions have higher mitochondrial NOS (nNOS α isoform), indicating that mitochondrial NOS is physically close to the complex I [49].

The conflicting results regarding the existence and identity of mtNOS can be explained by several methodological issues, which have been discussed in detail by Brookes P.S. [50]. Some of these problems include mitochondrial purity, the presence of other cell types in tissues (eg, vascular endothelial cells, inflammatory cells), the detection of the mitochondrial NO production, and the immunodetection of NOS isoforms. If mtNOS does exist despite methodological problems, the identity of mtNOS could also be related to the tissue of origin, which could explain the inconsistency in the identified isoform, for example, mtNOS could be an isoform of neurons in the brain, while iNOS could be in the brain and liver.

The existence of mtNOS can be confirmed by the relevance of NO in the control of mitochondrial respiration, since, when produced in mitochondria, NO will have a quick and easy access to its target, cytochrome oxidase, which is inhibited by NO [51]. However, it is also argued that, given the high diffusivity of NO, it is not necessary that it be produced near its site of action [52]. For example, NO can be produced by eNOS in vascular endothelial cells or by eNOS located on the surface of the cytosol of the outer mitochondrial membrane, then diffuse through other compartments reaching the respiratory chain [52].

Nitric oxide receptors

The most important physiological signaling pathway stimulated by NO, is the activation of soluble guanylate cyclase and the formation of cyclic guanosine monophosphate (cGMP). Soluble guanylate cyclase (sGC) is a key protein in the regulation of many physiological functions in mammals. This is a major NO receptor that plays a central role in the BP regulation, wound healing, memory formation, and other key physiological processes. The involvement of rHC is oftener and oftener found in various pathophysiological processes [53].

Since the rate constant of the NO association with heme centers is usually very high compared to other potential targets, even low, nanomolar, concentrations of NO are sufficient to activate sGCs. Rapid reactions between NO and targets with such kinetics limit alternative signaling processes that may otherwise be detrimental to cell functions. Upon the NO stimulation, sGC generates cyclic guanosine monophosphate from guanosine-5'triphosphate and activates protein kinase G-dependent signaling, required for various signaling cascades in several cell types. It is well known that dysregulation of sGC signaling leads to dysfunction of the cardiovascular, nervous, and gastrointestinal systems [54].

The physiologically significant mammalian sGC heterodimer consists of $\alpha 1$ and $\beta 1$ subunits and is expressed in most cell and tissue types; however, two other sGC subunits, $\alpha 2$ and $\beta 2$, have been identified, and mixed heterodimers have been discovered, in particular $\alpha 2\beta 1$, which is expressed in the brain, placenta, spleen, and uterus.

Both α and β subunits contain the cyclase homology domain and belong to the class III cyclase family. Since the active site of sGC is located at the heterodimer boundary, two active centers are possible, but when subunits are connected, catalytic and pseudosymmetric pockets are formed [55].

Various physiological studies have shown that the $\beta 1$ subunit of the heterodimer is necessary but not sufficient for proper sGC signaling; both $\alpha 1$ and $\alpha 2$ subunits can heterodimerize with the $\beta 1$ subunit to form a functional protein. The conservative histidine on the $\beta 1$ subunit was found to coordinate the heme moiety, which preferentially binds NO and carbon monoxide (CO) rather than oxygen (O_2). Structural changes caused by the NO binding, activate the catalytic domain of the sGC protein, which causes vasorelaxation [56].

Elucidation of the NOS role in physiological and pathophysiological processes in the body NOS uncoupling as a basis for pathophysiological conditions

Under physiological conditions, eNOS produces NO, which is a key vasoprotective factor in the endothelium.

However, under pathological conditions associated with an oxidative stress, eNOS may cease to function. The oxidative stress contributes to endothelial dysfunction primarily due to the rapid inactivation of NO via oxidation by excess superoxide. At the second stage, a constant oxidative stress renders eNOS unbound, whereby superoxide is produced at the expense of NO. The deficiency of the eNOS cofactor tetrahydrobiopterin (BH_4), the deficiency of the eNOS substrate, L-arginine, and S-glutathionylation of eNOS are likely the main causes of the eNOS uncoupling. Peroxynitrite and superoxide can oxidize BH_4 leading to the BH_4 deficiency. The production of ROS from unbound eNOS has been shown in mouse models of atherosclerosis, as well as in patients with hypercholesterolemia, atherosclerosis, hypertension, diabetes mellitus, and in people who use nicotine for a long time [57].

Under conditions of atherosclerosis and vascular diseases, the bioavailability of NO in the vasculature decreases. All the known risk factors for cardiovascular diseases are accompanied by a decrease in NO bioavailability as a result of a decrease in its production and an increase in the superoxide inactivation.

Thus, a decrease in the NO production is not the result of a decrease in the eNOS expression. On the contrary, the eNOS expression is compensatory increased in vascular diseases. However, this compensation mechanism is often useless because the eNOS activity is inhibited or the eNOS enzyme becomes uncoupled (inoperative) [58].

Molecular mechanisms of NOS uncoupling

The most significant cause for NOS uncoupling is the loss of the critical cofactor BH_4 . It can be synthesized in two ways: in a three-step process from guanine triphosphate (GTP) to BH_4 and the so-called "rescue route", which recycles oxidized 7,8 dihydrobiopterin back to BH_4 using the recycling enzyme dihydrofolate reductase (DHFR, dihydrofolate reductase). An oxidative stress plays a significant role in the depletion of cellular BH_4 pools, leading to uncoupling. Superoxide can directly oxidize BH_4 to dihydrobiopterin (BH_2) and, therefore, destabilize the NOS dimer, leading to uncoupling. This appears to be the main reason for NOS uncoupling during hypertension and ischemic reperfusion, as increased superoxide levels correspond to a decrease in the BH_4/BH_2 ratio and subsequent NOS uncoupling. The oxidative stress can also affect the bioavailability of BH_4 not only through oxidation, but also through a direct decrease in the DHFR expression, thereby reducing the activity of the "rescue pathway" [59].

A direct oxidation of BH_4 by superoxide is relatively slow, but BH_4 is rapidly oxidized to BH_2 by peroxynitrite, a

highly reactive anion formed by the reaction of superoxide with NO. Peroxynitrite also causes significant cell damage by nitrating the thiol and hydroxyl groups of the amino acid side chain, thereby impairing the enzymatic function.

The consequences of NOS uncoupling include: reduced the *de novo* NO formation; binding of bioactive NO by superoxide anions through the formation of peroxynitrite; cellular damage mediated by superoxide and peroxynitrite; the peroxynitrite mediated oxidation of BH_4 to BH_2 leading to a further propagation of NOS uncoupling.

Thus, NOS uncoupling is a self-reinforcing biochemical process driven by BH_4 oxidation. Due to the unbound eNOS-dependent oxidative stress, BH_4 oxidation has been observed both *in vitro* and *in vivo*. In these experiments, uncoupling was induced by increasing the eNOS expression under the conditions of a stable expression of GTP-cyclohydrolase (GTPCH-I), effectively reducing the ratio of BH_4 to eNOS. If all other variables are held constant, the resulting oxidation of BH_4 to BH_2 can only be due to the formation of superoxide by the decoupled NOS. Cellular superoxide is also produced by a number of other mechanisms, including NADPH oxidase, xanthine oxidase, and redox processes. In pathology, the oxidative stress can induce BH_4 oxidation, thereby initiating or maintaining NOS uncoupling. Excess superoxide resulting from the initial BH_4 oxidation and NOS uncoupling will stimulate a cycle of cellular damage, a further BH_4 oxidation (BH_4 oxidation stabilizes unbound NOS subunits that continue to produce ROS and biochemical stabilization of the unbound NOS subunit activity). The interruption of this communication process is a promising strategy for the treatment of a wide range of cardiovascular diseases caused or exacerbated by the NOS uncoupling (Fig. 2) [60, 61].

Peroxynitrite leads to the NOS uncoupling. Although peroxynitrite can directly oxidize BH_4 *in vitro*, it can also directly affect the enzyme itself, which also leads to uncoupling: peroxynitrite releases zinc from the zinc-thiolate cluster and destroys the NOS dimer (Fig. 3) [59].

S-glutathionylation is a reversible post-translational modification that adds glutathione to reduced cysteine residues. This most often occurs during the oxidative stress, as eNOS acts as one of the main targets for S-glutathionylation. It has been found out that the eNOS-dependent production of NO and superoxide is modulated by its S-glutathionylation, indicating that intense S-glutathionylation correlates with a decrease in the NOS activity and an increase in the superoxide production. The increased oxidative stress causes a proportional increase in the amount of S-glutathionylated eNOS, which also indicates that the production of superoxide by the upregulation principle is a trigger for the eNOS uncoupling [59].

Thus, S-glutathionylation uncouples eNOS, switching it from NO to the ROS formation, by attaching glutathione to cysteine residues between the FMN-binding and FAD-binding domains: this leads to enzyme uncoupling and the production of O_2^- free radicals. The oxidative stress triggers S-glutathionylation of eNOS in endothelial cells and intact vessels. In addition, S-glutathionylation is increased during vasoconstriction, which reduces endothelium-dependent vasodilation. Given the importance of NO and eNOS mediated endothelial dysfunction in diseases such as heart attack, stroke, diabetes and cancer, the identification of this new redox signaling pathway provides new insights into therapeutic approaches to prevent or treat them [62].

Drugs for prevention of NOS uncoupling

As it has been noted above, NOS uncoupling is one of the main factors in the development of various diseases and, therefore, a promising target for the pharmacological intervention. Many approaches have been taken to try restoring NOS in various diseases, including: blocking the upstream of the ROS production, the BH_4 administration, the administration of folic acid to recycle BH_2 to BH_4 , and the L-arginine administration (Fig. 4).

BH_4

A decreased BH_4 bioavailability followed by the eNOS uncoupling has a significant impact on the pathogenesis of endothelial dysfunction, which is a hallmark of vascular damage in cardiovascular pathologies, including hypertension, hyperlipidemia, and diabetes [63]. The intracellular ratios of BH_4 /eNOS (and of BH_4 and other NOS isoforms) remain poorly defined, making it unclear whether the intracellular BH_4 deficiency is sufficient to induce the eNOS uncoupling. The BH_4 / BH_2 ratio and the absolute molar concentration of BH_4 are key determinants of eNOS binding *in vivo*. The degree of BH_4 oxidation, BH_2 accumulation, and the superoxide production are directly correlated with the intracellular eNOS/ BH_4 ratio. Through the mechanisms independent of eNOS, such as a direct uptake of reactive oxygen species, BH_4 can have a significant impact on the levels of the reactive oxygen species production by cells [64].

Thus, the replenishment of intracellular BH_4 can be considered a promising approach to stabilize NOS. However, there are reasons that limit the clinical application of this approach. The oral administration of excess BH_4 may be not effective if the NOS isoforms ratio is not balanced: too much BH_4 can activate iNOS under the conditions of inflammation, exacerbating the pathophysiology of this process. BH_4 administered p. o., is likely to oxidize and needs to be regenerated, but the attempts to enhance this re-regeneration process by combining BH_4

with antioxidants (vitamin C) have not resulted in the improved clinical efficacy of this combination. BH_4 has already been approved by the Food and Drug Administration (FDA, <https://www.fda.gov/>) as a therapeutic agent for the treatment of phenylketonuria, a phenylalanine hydroxylase deficiency that can be partially corrected by the administration of BH_4 . Sapropterin dihydrochloride (Kuvan®, BioMarin, Tiburon, CA, www.vidal.ru) is a synthetic version of BH_4 currently used to treat patients with phenylketonuria [65, 66].

An analog of BH_4 is the compound 6-acetyl-7,7-dimethyl-5,6,7,8-tetrahydropterin (ADDP), a stable compound, soluble in both polar and organic solvents. ADDP can diffuse from plasma through cell membranes and cause vasodilation by stimulating the eNOS activity [67].

Metformin can attenuate the oxidative damage to endothelial cells by increasing the levels of GTP cyclohydrolase-I (GTPCH-I) and BH_4 , and thereby preventing the eNOS uncoupling. Metformin also inhibits the p47-phox subunit of NADPH oxidase, thereby reducing the ROS production and BH_4 oxidation. These protective effects of metformin are partially mediated by the activation of the adenosine monophosphate-activated protein kinase (AMPK) signaling pathway [68].

Arginase inhibitors

The role of the arginase overexpression and/or activity and its downstream targets in cardiovascular dysfunction and injury, has been well established. Pharmacological effects on specific components of the arginase/ornithine pathway are promising as therapy for cardiovascular diseases [69].

Ureohydrolase arginase is a manganese-containing enzyme that catalyzes the final step of the ornithine cycle by converting L-arginine to L-ornithine and urea. The two isoforms of this enzyme have similar mechanisms of action and produce the same metabolites. The homology of their amino acid residues is more than 60%, and the regions important for the function of the enzyme are 100% homologous. In addition, both isoforms are involved in the dysregulation of the NOS function [70].

The arginase-induced uncoupling of eNOS leads to the increased ROS production, which contributes to the endothelial dysfunction and increased vascular stiffness. Increasing the level of arginase can reduce the concentration of L-arginine and has been shown to contribute to the pathophysiology of the circulatory system diseases [71].

Preclinical studies have shown that the addition of L-arginine can improve the erectile dysfunction, prevent or reduce the severity of the endothelial dysfunction. However, some studies on animals and humans have found out no benefit or increased adverse effects from

the chronic administration of L-arginine. The resulting side effects are likely related to the counter-regulatory effects of L-arginine in the increasing arginase expression/activity, which may reduce the availability of L-arginine for NOS. Under the conditions of the excessive arginase activity, a competition with NOS for L-arginine causing the enzyme uncoupling, will occur. The increased formation of reactive oxygen species and key inflammatory mediators contributes to a pathological increase in the arginase activity [71].

Thus, several amino acids inhibit arginase, including: L-ornithine, L-leucine, L-valine, L-lysine, L-isoleucine, and L-norvaline. L-ornithine is the most potent of the above-listed. In addition, L-citrulline increases the NO production, but is also an allosteric inhibitor of arginase [71].

The research by El Bassossy H.M. et al. showed that the inhibition of arginase by citrulline, norvaline, or ornithine, alleviates hypertension associated with a metabolic syndrome by direct and indirect defense mechanisms. The direct mechanism is to maintain an endothelium-dependent relaxation and the NO generation, while the indirect mechanism is realized through the inhibition of insulin resistance and hypertriglyceridemia [72]. Ornithine inhibits the enzyme arginase by competing with its substrate (L-arginine) at the active sites of the enzyme. L-norvaline is a noncompetitive inhibitor of the enzyme arginase, and L-citrulline is its allosteric inhibitor.

L-norvaline (or 2-aminopentanoic acid) is a non-proteinogenic amino acid and an isoform of the common amino acid valine that has been extensively investigated in early enzymological studies. The structural similarity with L-ornithine gives the substance the ability to inhibit arginase by the negative feedback principle. In addition, the anti-inflammatory properties of L-norvaline have been demonstrated *in vitro* through the inhibition mechanism of ribosomal protein kinase S6-kinase $\beta 1$. It should be notified that the arginase inhibition is promising for reducing the risk and frequency of cardiovascular diseases [73].

Statins inhibit HMG-CoA reductase, which catalyzes the conversion of HMG-CoA to mevalonic acid, limiting cholesterol biosynthesis in the liver. In addition to inhibiting cholesterol synthesis, statins also block the synthesis of intermediate isoprenoids such as farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP). FPP and GGPP serve as important lipid components for post-translational modification of various proteins, including heterotrimeric G proteins and small GTP-binding proteins belonging to the Ras, Rho, Rap, and Rab family of GTPases. Isoprenylation is critical for the intracellular transport and the function of small GTP-binding

proteins. The modification with FPP is required for a proper localization of Ras family proteins, while GGPP is required for Rho, Rab and Rap family proteins. However, some Rho GTPases require both farnesylation and geranylgeranylation for proper functioning and the intracellular localization. By inhibiting the synthesis of mevalonate, statins inhibit the synthesis of intermediate isoprenoids, thereby preventing the isoprenylation of small GTPases, which leads to the inhibition of these signaling molecules. Interestingly, some of the cholesterol-independent or "pleiotropic" effects of statins may be related to the ability of statins to block the synthesis of intermediate isoprenoids [74, 75].

The ability of statins to increase the eNOS expression and activation may be an important mechanism by which statins improve the endothelial function and lower cholesterol levels (Fig. 5).

Geranylgeranylation of Rho GTPase has been shown to result in the downregulation of eNOS in endothelial cells; the administration of statins inhibited the formation of GGPP and therefore resulted in an increase in the eNOS expression, which was not observed during the co-incubation with GGPP. It has also been shown that an increased level of endogenous low-density lipoproteins (LDLs) negatively affects the expression of eNOS mRNA in human umbilical vein endothelial cells (HUVECs), and this effect, at the post-transcriptional level, was neutralized after the administration of simvastatin. The mechanism by which statins increase the eNOS mRNA stability is through an increased polyadenylation of eNOS mRNA due to the changes in the actin cytoskeleton caused by the Rho inhibition. In addition, the statin treatment of HUVECs induced by hydrogen peroxide (H_2O_2) activates the phosphatidylinositol 3-kinase (PI3K, phosphoinositide 3-kinase)/Akt pathway and enhances the eNOS expression. Tumor necrosis factor alpha ($TNF\alpha$) is a powerful inflammatory stimulus that activates miR-155 miRNA and, accordingly, eNOS mRNA. It is important to notify, that its ($TNF\alpha$) activation was attenuated by simvastatin, but the positive effect of the statin was abolished by co-incubation with mevalonate or GGPP [76].

In addition to increasing the eNOS expression at the transcriptional and post-transcriptional levels, at the post-translational level, statins enhance the activity of eNOS, which has multiple phosphorylation sites by serine/threonine residues. A HUVEC exposure to fluvastatin increases eNOS phosphorylation at the Ser-633 and Ser-1177 activation sites via protein kinase A (PKA) and the PI3K/Akt-mediated pathway, respectively. The statin-mediated induction of eNOS phosphorylation at Ser-1177 depends on the recruitment of Akt to the eNOS complex in endothelial cells (heat shock protein-90). In endothelial cells, atorvastatin increases eNOS phosphorylation

at Ser-633 via adenosine monophosphate-activated protein kinase (AMPK). Moreover, the *ex vivo* incubation of rat mesenteric resistant arteries with simvastatin, induces rapid AMPK-mediated eNOS phosphorylation at Ser-1177. In addition to modulating phosphorylation, statins also increase the eNOS activity at the post-translational level, causing it to dissociate from caveolin-1. Atorvastatin reduces the content of caveolin-1 and activates eNOS in EC, regardless of the presence or absence of LDL cholesterol [77].

Statins act at several levels, increasing both eNOS activity and stabilizing its dimers. The exposure of HUVEC to cerivastatin or fluvastatin results in the increased GTPCH expression and the increased BH₄ bioavailability, resulting in improved eNOS binding. In addition, statins prevent the oxidation of tetrahydrobiopterin to trihydrobiopterin (BH₄ to BH₃). The result of these processes is a decrease in the oxidative stress in endothelial cells and an improvement in the eNOS function [76].

Asymmetric dimethylarginine (ADMA) acts as an endogenous inhibitor of eNOS. ADMA is produced as a by-product of protein metabolism due to methylation of L-arginine residues, its production is significantly increased by LDL in endothelial cells, and it is metabolized by the enzyme dimethylarginine dimethylaminohydrolase (DDAH); under physiological conditions, it is excreted in the urine. Due to its close structural similarity to L-arginine, ADMA suppresses the eNOS activity either through the inhibition of the L-arginine cellular uptake or through a direct competitive inhibition of eNOS binding [78].

There are several hypotheses about the ways statins affect ADMA levels. One of them concerns the inhibition of the ADMA-induced inflammatory response modulated by mitogen-activated protein kinase (MAPK) in human endothelial cells. Statins activate the transcription factor of sterol response element binding protein (SREBP) by lowering membrane cholesterol. This factor specifically enhances the expression of more than 30 genes associated with the synthesis and absorption of fatty acids, phospholipids, cholesterol and triglycerides. One of its isoforms, a sterol regulatory element binding protein 2 (SREBP-2), increases the transcription of proprotein convertase subtilisin/kexin type 9 (PCSK9, proprotein convertase subtilisin/kexin type 9). Statins increase both PCSK9 mRNA and LDLR levels by activating sterol-mediated SREBP-2, an important activator of the transcription and DDAH activity (Fig. 6) [79].

Resveratrol

Resveratrol (3,5,4'-trihydroxy-trans-stilbene) is a phytoalexin polyphenol found in various plant species including *Veratrum grandiflorum* (Maxim. ex Baker) O.

Loes, *Polygonum cuspidatum* Siebold & Zucc., *Vitis vinifera* L., *Arachis hypogaea* L. and *Morus rubra* L. The name "resveratrol" comes from its origin; the compound is a resorcinol derivative from the species of the genus *Veratrum*.

Molecular targets of resveratrol include those which interact directly with it and the others that are modulated indirectly (e.g., through changes at the expression levels). For its effect on eNOS, the following targets are of particular importance: NAD⁺ – dependent histone deacetylase sirtuin 1 class III (SIRT1, silent information regulator 1); AMP-activated protein kinase (AMPK); nuclear factor-derived erythroid 2 – dependent factor-2 (Nrf2, nuclear factor E2-related factor 2); estrogen receptors (ER).

A part of the resveratrol effects on the cardiovascular system is mediated by endothelial NO. Resveratrol enhances the NO production through several mechanisms and prevents the NO breakdown by reducing the oxidative stress (Fig. 7).

Activators of various NOS isoforms

Calcium dobesilate (Doxium 1)

Calcium dobesilate (CaD) is widely used as an angioprotective agent for the treatment of vascular diseases, inhibits platelet aggregation, thrombus formation, increased capillary permeability, reduces their fragility, increased blood viscosity.

A specific property of dobesilate is a moderate increase in the eNOS activity with little or no effect on the iNOS activity, which indicates its future outlook for the treatment of vascular diseases, especially those caused by impaired NO secretion in the endothelium, including prolonged hyperglycemia [81].

The mechanism of calcium dobesilate action on the eNOS expression is realized in several ways.

As a result of the study of the molecular and cellular mechanisms underlying the protective effects of CaD carried out by Zhou Y. et al. [84], the effect of calcium dobesilate on pentraxin 3 (PTX3) was established. This protein is an important component of humoral immunity and is secreted by various cells, including endothelial, mononuclear macrophages, fibroblasts, adipocytes, dendritic and smooth muscles after the stimulation of the inflammatory response. High plasma levels of PTX3 are associated with many diseases conjugated with endothelial dysfunction (diabetes mellitus, arterial hypertension, chronic kidney disease). PTX3 interferes with eNOS phosphorylation by serine 1177, reducing NO production, leading to endothelial dysfunction.

PTX3 induces dysfunction and morphological changes in the endothelial layer via the P-selectin/matrix metalloproteinase-1 pathway, which prevents the sep-

aration of eNOS from caveolin-1, resulting in impaired NO signaling [82]. Another study also confirms that PTX3 inhibits the NOS/NO pathway by inhibiting eNOS phosphorylation and therefore not inducing a detachment from caveolin-1 required for activation [83].

Thus, calcium dobesilate can inhibit the PTX3 expression by altering the IKK/I κ B/NF- κ B pathway, thereby improving endothelial dysfunction at the cellular level [84].

Endothelial cells also need a vascular endothelial growth factor A (VEGF-A) to maintain their various functions (differentiation, permeability, and the NOS expression) and survive [85], however, an excessive amount of this factor causes their damage, increased glomerular permeability and inflammation. An appropriate balance of the VEGF expression, binding, and signaling is required to maintain glomerular health and prevent glomerulosclerosis and rarefaction. Under pathological conditions, this balance is disturbed [86].

Despite high concentrations of VEGF in the vasculature, a reduced NO response was called "uncoupling" of eNOS and VEGF, which leads to the disruption of the cellular response and the intensification of the pathological process course. In addition to its positive effect on eNOS, at the molecular level, calcium dobesilate also reduces the oxidative stress and inhibits growth factors such as fibroblasts growth factor (FGF) and VEGF [87].

CavNOxin

CavNOxin is a synthetic peptide of 20 amino acids in the framework domain of caveolin with a triple alanine substitution fused to the peptide penetratin for a free entry into cells.

CavNOxin increases NO levels, decreases a vascular tone, and lowers mean BP in wild-type mice, but the effect is lost in gene or caveolin-1 knockout animals, indicating that it is caveolin-1 specific.

A chronic CavNOxin administration reduces the severity of atherosclerosis in mice with apolipoprotein E (ApoE, apolipoprotein E) knockout, fed a high-fat diet, and in mice with diabetes and atherosclerosis, while the loss of eNOS genetically reduces its effect. These results suggest that CavNOxin may reduce the inhibitory effect of caveolin on the eNOS function, allowing increased endogenous NO biosynthesis. The data from genetic studies using transgenic mice with the endothelial-specific expression of F92A CAV1 confirm that the regulated expression of modified caveolin is sufficient to increase the NO bioavailability, which leads to a significant decrease in BP [88].

NOS transcription enhancers

AVE3085 and AVE9488 are two small molecules that

have been identified by high-throughput screening able of enhancing the eNOS67 transcription *in vitro* and *in vivo*.

A promoter analysis using the luciferase test showed that both compounds bind even the shortest fragment of the eNOS promoter (263 polynucleotides), but do not overlap with known eNOS transcription factors (GATA, Sp1/3-like, Elf-1, YY1, Sp1 or PEA3).

AVE3085 has a promoter binding cis element located in the proximal 263 base pairs of the promoter region.

In the experiments on the human internal mammary artery obtained after a coronary artery bypass surgery, a positive effect of AVE3085 was shown. It was expressed in the prevention of the damaging effect of homocysteine on the endothelium during the co-incubation with AVE3085, while an increase in the level of eNOS mRNA and an increase in the NO production was observed [89].

These NOS transcription enhancers have also been shown to increase vascular BH₄ levels, reduce a cuff-induced neointima formation, and improve the course of atherosclerosis in ApoE knockout animals after 12 weeks of diet. AVE9488 promotes cardiomyocyte remodeling in a model of myocardial infarction, which can be explained by a decrease in SMAD-mediated signaling [90].

Blockers of various NOS isoforms NOS inhibitors can be classified from different points of view.

A classical enzymological approach makes it possible to distinguish reversible (subgroups: competitive, non-competitive and mixed type), irreversible, as well as reactive inhibitors, the action of which depends on the enzymatic reaction.

The most widely used classification of inhibitors is based on the site of their binding to the NOS enzyme, which makes it possible to recognize four different classes of inhibitors.

The first class, in fact the largest, interacts with the arginine-binding site. Some of the compounds belonging to this class are reaction-level inhibitors because they require an active enzyme and NADPH for a complete inhibition. The second class includes a set of compounds that mimic the BH₄ cofactor. The third class is represented by inhibitors that directly interact with heme. Some inhibitors belonging to this class bind to the heme of the monomeric form of the enzyme and prevent the formation of its active dimer. The fourth class covers NOS inhibitors interacting with calmodulin or flavin cofactors [91].

There is also a classification according to which NOS inhibitors can be divided into two groups: based on amino acids (derivatives and close analogues of arginine) and based on non-amino acids (a wide range of

compounds that do not have an arginine-like amino acid backbone). Arginine-based NOS inhibitors have generated a particular interest as this category includes a large number of compounds with potential experimental and clinical applications. Since these compounds are readily available and stable in the aqueous media, they serve as an excellent tool for an experimental inhibition of NOS [91].

Non-selective inhibitors

Even before crystal structures had been created, arginine-based compounds were found to inhibit NOS and exhibit neuroprotective properties. For example, NG-mono-methyl-L-arginine (2 L-NMMA) and NG-nitro-L-arginine methyl ester (3 L-NAME) protect brain neurons in a stroke and Parkinson's disease. However, these inhibitors also cause hypertensive effects, most likely due to the inhibition of eNOS.

The earlier studies have shown that L-arginine substrate analogs have inhibitory properties against three NOS isoforms. Among them, NG-monomethyl-L-arginine (L-NMMA) and NG-nitro-L-arginine (L-NNA) and its precursor NG-nitro-L-arginine methyl ester (L-NAME) have been characterized as common NOS inhibitors and continue to be widely used in research, especially L-NAME [92].

Endogenous ADMA and L-NMMA inhibitors

There are two endogenous NOS inhibitors. ADMA is a potent, non-competitive NOS inhibitor, while its afined L-NMMA is a less potent, competitive NOS inhibitor. Although ADMA has been shown to contribute to the development of the inflammatory syndrome and endothelial dysfunction observed in shock, the possibility of its clinical application requires a further study [91].

546C88 (N(G)-methyl-L-arginine hydrochloride)

Compound 546C88 (N(G)-methyl-L-arginine hydrochloride) is a non-selective NOS inhibitor that appears to rebalance a vasomotor tone in patients with septic shock, by reducing the concomitant need for norepinephrine. It has been studied in phase III clinical trials in Europe, North America, South America, South Africa and Australia. This study was terminated in advance due to an increased risk of death [91].

L-NAME

N-nitro-L-arginine methyl ester (L-NAME) and Ng-nitro-L-arginine (L-NArg) are synthetic, non-selective NOS inhibitors with implications for a substance abuse because they attenuate signs of opioid withdrawal in rats. L-NAME also seems promising for the treatment of a septic shock by maintaining adequate BP levels. A

chronic administration of L-NAME reduces angiogenesis during *in vitro* migration and invasiveness, pointing to its possible future use as a tumor growth inhibitor [91].

VAS203

Pterin 4-amino-tetrahydrobiopterin (VAS203) is a BH₄ analogue that can inhibit all NOS proteins by replacing the BH₄ cofactor. VAS203 has shown efficacy in the treatment of the traumatic brain injury and has been used in phase II clinical trials [92].

Selective inhibitors of iNOS

N-[3-(aminomethyl)benzyl]acetamide

N-[3-(aminomethyl)benzyl]acetamide is a specific iNOS inhibitor. It remains one of the most widely used NOS inhibitors in the research due to its cell and tissue permeability. Its selectivity appears to be associated with an irreversible effect on the more rapidly responsive iNOS, while the inhibition of nNOS and eNOS is reversible. The similar effect is observed in N(5)-(1-iminoethyl)-1-ornithine (1-NIO) [92].

7-nitroindazole

One of the first highly selective compounds is 7-nitroindazole (7-NI). Initial crystallographic studies showed that 7-NI binds at the active site of eNOS and changes the Glu orientation of the active site, while 3-bromo-7-NI can bind at both the active and pterin sites [93]. 7-nitroindazole is a specific nNOS inhibitor and exhibits an anticonvulsant effect on models of seizures in rodents. It has also been shown to occasionally induce convulsions in rodents caused by cainite, nicotine, and soman. Although it has been shown that all three isoforms can bind 7-NI with very similar affinity, *in vivo* studies have shown inhibition of nNOS without significant effect on BP. This may be due to the fact that 7-NI can be taken up by cells in different ways and the permeability of endothelial cells for this compound is limited. Thus, although 7-NI cannot be accurately described as a specific inhibitor of nNOS, it may behave as such in *in vivo* models [92].

Aminoguanidine

Aminoguanidine is a selective iNOS inhibitor that attenuates the "graft versus host" disease by reducing hematopoietic parameters and a concomitant susceptibility to the bacterial infection in mice [94].

L-NIL

An exceptional compound among the first arginine-based NOS inhibitors developed is L-NIL. It has a moderate selectivity for iNOS, is well tolerated, and its prodrug (L-NIL-TA) can be administered orally [95].

GW273629 и GW274150

Compounds GW273629 and GW274150 are sulphur-substituted acetamidine amino acids that are specific iNOS inhibitors. Having safer toxicity profiles than 1400W, GW273629 and GW274150 have been used in clinical trials for migraine therapy (NCT00242866; NCT00319137). The both compounds were found to be ineffective, possibly due to the peculiarities of pharmacokinetics [91, 92, 95].

eNOS

As mentioned above (see section “Endothelial NO synthase”), eNOS is posttranslationally palmitylated and transported to caveolae, where its activity is reduced due to binding to caveolin-1. Based on this mechanism, it seems possible to mimic the function of caveolin-1.

Based on this approach, cavtratin, a drug that inhibits eNOS, was developed. It is a caveolin scaffold domain fused to the cell-permeable peptide penetratin, which allows caveolin to pass through the plasma membrane. Cavtratin significantly targets at eNOS, reducing its activity and mimicking the inhibitory effect of caveolin-1, while not affecting the function of other NOS *in vivo*. Cavtratin reduces an increased microvascular permeability and blocks a tumor progression (shown in the model of HepG2 hepatocarcinoma cells transplanted into mice without thymus), restores tight junctions in brain microvascular endothelial cells treated with chemokine-2, inhibits matrix metalloproteinase-2/9 and cyclooxygenase-2, which can lead to the stabilization of atherosclerotic plaques and reduce the risk of intracerebral hemorrhage. Cavtratin also reduces the growth of glial cells and has a protective effect on the blood-brain barrier when exposed to VEGF-A in a mouse model of multiple sclerosis.

It remains unknown whether eNOS is the only target of cavtratin. However, most of the blocking effects of VEGF and the Transforming growth factor (TGF) were reversed when the experiments were performed in eNOS-deficient mice.

It is possible that cavtratin may increase blood pressure during a course administration. However, there is no evidence to support this potential effect. Furthermore, since CAV1 is believed to regulate many intracellular signaling pathways, cavtratin may have pleiotropic effects on many tissues, especially those lacking eNOS [90].

Other approaches to inhibition

Inhibition of downstream mediators

Methylene blue is a soluble guanylate cyclase (sGC) inhibitor. It is a dye that can be safely used in the people with a septic shock. It provides an effective protection in

the TNF α -induced shock as well as in anaphylactic hypotension not corrected by other pharmacological agents. Methylene blue may be also useful in refractory cases of vasoplegia, a common complication of cardiopulmonary bypass, by alleviating an inflammation-mediated dysregulation of the endothelial function [96]. 1H-[1,2,4]oxadiazolo-[4,3-a]quinoxalin-1-one (ODQ) is a selective sGC inhibitor in rats that restores a basal ganglia function and improves motor symptoms in Parkinson's disease [94].

NO recycling

An NO-sensitive hydrogel, which demonstrated its fast response and sensitivity to NO, was developed by the research group of Park J. et al. That presented a potential for the application in biology and medicine [96]. There are projects to evaluate the ability of NO-cleavable cross-linkers (NOCCLs) to capture NO for the treatment of rheumatoid arthritis (RA). This is the first study on the possibility of using endogenous NO directly for the treatment of RA. By controlling the concentration of acrylamide as a polymer base and NOCCL as a crosslinking agent, a spherical nanogel was prepared by a solution polymerization to obtain an NO-neutralizing nanosized hydrogel (NO-Scv gel) for treating rheumatoid arthritis. As expected, the NO-Scv gel took up NO and reduced the levels of pro-inflammatory cytokines produced by activated macrophages *in vitro*. In addition, after an intra-articular injection of the nanogel into each paw of the mice with experimental RA, the therapeutic effects observed were superior to those notified with *in vivo* dexamethasone [98], which inhibits an iNOS-dependent NO formation by destabilizing iNOS mRNA by a mechanism requiring *de novo* protein synthesis. The effect of dexamethasone on the iNOS expression and the NO production, as well as on the degradation of iNOS mRNA, is to some extent abolished by type II interferon [97].

Therapeutic applications of activity modulators of various NO synthase isoforms

After various NOS isoforms had been identified, the search for their inhibitors progressed rapidly. As more and more active molecules were becoming available, it has become clear that the selectivity for NOS isoforms is a major problem. All 3 isoforms of human NOS (nNOS, eNOS, and iNOS) have nearly identical active site structures, making it difficult to design a selective inhibitor. It is especially important to avoid inhibition of eNOS due to its vital role in the cardiovascular system.

Experimental approaches using modulators of nitric oxide synthesis, including substrate manipulations, NO synthesis donors and inhibitors, will be useful to establish the relationship between NO systems and the cells of the cardiovascular and renal systems.

As a vasodilator, NO may be a unique therapeutic agent for the treatment of hypertension and, as a result, renal failure and left ventricular hypertrophy. The inclusion of NO modulators in clinical practice may be useful not only as therapeutic agents for certain diseases, but also as means to slow the progression of diseases due to their influence on many systems of the mammalian body.

As a therapeutic approach, a modulation of the NOS activity can be implemented to treat endothelial dysfunction, which is the cause for many diseases. For example, the development of endothelial dysfunction is characteristic of diabetes mellitus: the course of this disease is characterized by a number of metabolic disorders, which ultimately lead to the disruption of functioning of various organs and systems, mainly cardiovascular. A summary of the characteristics of various NOS is presented in Table 1.

CONCLUSION

Enzymes of the NOS group became pharmacotherapeutic targets immediately after their key role in the NO biosynthesis had been established, and the interest in

them is growing as new aspects of the pathogenesis of many acute and chronic diseases are being elucidated. Currently, almost all human diseases are to some extent associated with the endothelium and its dysfunction, and the restoration of the adequate vascular homeostasis is impossible without blood flow maintaining in the microvasculature at the physiological level. Increasingly, therapeutic strategies for many socially significant diseases (metabolic, cardiovascular, and neurometabolic) are endotheliocentric in nature and are aimed at preventing primary or secondary cardiovascular complications. Today, approaches to the stimulation of angiogenesis factors are being studied for the treatment of cardiovascular diseases, which show promising results in animals, but have not yet been implemented in the clinic. One important reason for this is the complexity and a large number of related molecular mechanisms that regulate ischemic vascular remodeling. Being responsible for the coordination and activity of vascular factors, the NO molecule can be such a regulator. Therefore, the NOS activity modulation may thus serve as an important factor for the operation of the complex network, contributing to a greater efficiency of angiogenesis.

FUNDING

The work was supported by the Russian Science Foundation (Project No. 20-75-10013).

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Denis V. Kurkin, Elizaveta E. Abrosimova, Dmitry A. Bakulin, Evgeniy I. Morkovin, Ivan N. Tyurenkov – conception, article planning, review of literary sources, collection of materials, writing and editing the article; Nikolai S. Kovalev, Marina A. Dubrovina, Aleksandr V. Borisov, Andrey V. Strygin – collecting materials, editing the article.

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