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Nitric oxide (NO) - a wide-range modulator of life's processes. The role in pathomechanism of alcohol addiction and withdrawal symptoms

Tlenek azotu (NO) wszechstronny regulator procesów życiowych. Rola w patomechanizmie uzależnienia od alkoholu i przebiegu zespołu abstynencyjnego

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Abstract

Introduction and aim: The aim of this study was to analyze the available literature in terms of understanding the functions and mechanisms of endo-and exogenous nitric oxide (NO). Particular attention was paid to the involvement of nitric oxide donors in the pathogenesis of alcohol dependence and withdrawal syndrome.

Results and discussion: Nitric oxide was discovered in 1770. Numerous studies about NO biochemistry have been carried out since that time In 1998 Robert F. Furchgott, Luis Ignarro and Ferid Murad received the Nobel Prize in medicine for their research on the sequence of metabolic changes: L-arginine → NO. There are various mechanisms leading to the activation of individual NOS isoforms and thus to NO synthesis. Moreover, NO formed in the reaction catalyzed by different NOS, has various functions. Excessive endogenous NO is involved in the pathogenesis of many diseases, e.g. atherosclerosis, degenerative diseases of the nervous system, inflammations, autoimmune diseases and cancers. Deficient endogenous NO is implicated in the development of hypertension, preeclampsia, arteriosclerosis, hypercholesterolemia. Exogenous NO is administered to patients with venous atherosclerosis of the lower limbs, coronary disease and pregnancy-induced hypertension. An increase or a slight decrease in NO metabolites plasma concentration following 4-week abstinence may be a predictor of alcohol drinking relapse during the next five months.

Streszczenie

Wprowadzenie i cel: Celem pracy była analiza dostępnej literatury pod kątem poznania funkcji i mechanizmów działania endo- i egzogennego tlenku azotu (NO). Szczególną uwagę zwrócono na zaangażowanie donorów tlenku azotu w patomechanizmie uzależnienia od alkoholu i przebiegu zespołu abstynencyjnego.

Wyniki i dyskusja: Tlenek azotu (NO) został odkryty jako związek chemiczny w 1770 roku. Od tej pory powstało wiele prac nad biochemią NO. W 1998 roku Nagrodę Nobla w dziedzinie medycyny za prace nad ciągiem przemian metabolicznych: L-arginina → NO otrzymali: Robert F. Furchgott, Louis Ignarro i Ferid Murad. Istnieją rożne mechanizmy prowadzące do aktywacji poszczególnych izoform NOS i w rezultacie do syntezy NO. Również NO powstały w reakcji katalizowanej przez różne NOS, spełnia w organizmie rożne funkcje. Nadprodukcja endogennego NO bierze udział w patogenezie wielu chorób m.in. innymi: miażdżycy, chorób zwyrodnieniowych układu nerwowego, zapaleń, chorób autoimmunologicznych i raka. Niedoborowi endogennego NO przypisuje się m.in. powstawanie nadciśnienia, w przebiegu preeclampsji, artherosclerozy, hipercholesterolemii. Pacjentom podaje się egzogenny NO, co poprawia stan chorych z miażdżycą żył kończyn dolnych, z chorobą wieńcową, z nadciśnieniem indukowanym ciążą. Zwiększenie lub niewielkie zmniejszenie syntezy tlenku azotu w ciągu pierwszych 4 tygodni abstynencji może być czynnikiem prognostycznym przerwania abstynencji w ciągu kolejnych 5 miesięcy.

Key words: nitric oxide, mechanisms of NO action, alcohol addiction, withdrawal symptoms
Słowa kluczowe: tlenek azotu, mechanizmy działania NO, uzależnienie od alkoholu, zespół abstynencyjny

Introduction

Nitric oxide (NO) is a wide-range modulator of many processes occurring in human and animal organisms.

It was discovered by Joseph Pristley (the discoverer of other 8 gases, including oxygen) in 1770 [1,2,3].

In 1977 Freid Murand examining the muscular membrane of the vascular wall noticed that vasodilators such as nitroglycerin, sodium nitroprusside act through nitric oxide which is their active metabolite [4].

In the same year Katsuki and colleagues [5] demonstrated that sodium nitroprusside, nitroglycerin resulted in increased cyclic GMP in various tissues. They observed that NO produced cGMP by activating the enzyme of guanyl cyclase (cGMP - a secondary transmitter, which has been known since 1963).

Numerous studies about NO biochemistry have been carried out since that time.

In 1980 Robert Furchgott and John Zawadski [4] examined the rabbit's aorta and observed that the relaxation of vessels induced by acethylcholine required the involvement of the endothelium.

Furchgott [6,7] named this factor – the endothelium-derived relaxing factor (EDRF).

Seven years later (1987) Luis Ignarro and Salvador Moncada [4] identified an easily diffusing particle produced by the endothelium, which, while acting as a synaptic transmitter on smooth muscles of the circulatory system, dilated vessels. That was nitric oxide (NO) [8].

In 1992 nitric oxide was hailed as a particle of the year by "Nature" journal.

In 1998 Robert F. Furchgott, Luis Ignarro and Ferid Murad [6,9] received the Nobel Prize in medicine for their research on the sequence of metabolic changes:

$$\text{L-arginine} \rightarrow \text{NO}$$

Today NO is known to be produced in the organism not only by the endothelial cells [10] but also by macrophages, hepatocytes, nerve endings and some neurons [11], neutrophils, monocytes, mastocytes, blood platelets [12].

Apart from N_2O (laughing gas) and NO_2 (air pollutant), NO is one of seven known nitric oxides.

NO is a colourless gas which, without enzymes activity, readily oxidizes to NO_2^-. NO_2^- is converted into nitrate being the final NO metabolite excreted in urine.

NO is produced in the organism from L-arginine with the help of nitric oxide synthase (NOS), also called digoxygenase [7,13].

$$\text{L-arginine} + \text{NADPH} + O_2 \xrightarrow{\text{NOS}} \text{L-citruline} + \text{NADP} + H_2O + \text{NO}$$

This is a two-step reaction of L-arginine oxidation in which NADPH is the source of electrons while L-citruline and NO are the products [13].

In the endothelial cells, L-cyruline reacting with L-aspartic acid leads to resynthesis of L-arginine. This process inhibits L-glutamic acid [14].

Five isoforms of NOS have been discovered: brain (bNOS), endothelial (eNOS), macrophage (macNOS), hepatocyte (hepNOS), mitochondrial (mtNOS) one. The studies on their structure and regulation are being conducted.

- The endothelial isoform (eNOS, NOS-1) and brain isoform (also called neuronal) (bNOS, nNOS, NOS-3) are constitutional isoforms (cNOS). The regulation of their activity occurs at the activation level. They depend on the calcium-calmoduline complex [12].
- The macrophage (macNOS) and hepatocyte (hepNOS) isoforms are connected with calmoduline and are independent of calcium ions, which are inducible (iNOS, NOS-2) by immune processes. The regulation of their activity occurs mainly at the level of gene expression [12,15].
- The mitochondrial isoform is poorly known. It is located on the internal protein-lipid membrane of mitochondria.

Some authors [16] claim that the only induced isoform is hepNOS.

- cNOS are constantly present in cells and NO synthesis catalyzed by them is short-lasting and pulsating so the concentrations of produced NO are low.
- iNOS are not present in cells unless their genes are expressed. This expression depends, among other things, on several cytokines. iNOS synthesize high concentrations of NO for a long period of time. The iNOS activity lasts as long as the substrate, i.e. L-arginine, is available-until the cell dies.

The sequence of all NOS is similar to that of cytochrome P450 reductase (CPR). NOS have the binding sites for: FMN, FAD, NADPH, calmodulin, tetrahydrobiopterin (BH4) [16,17].

Aim, material and methods

In the current study we studied references to know main functions of endogenous and exogenous nitric oxide (NO). We wanted to analyze mechanisms of NO action.

We focused our attention on nitric oxide (NO) involved in pathomechanism of alcohol addiction and withdrawal symptoms.

Results and Discussion

There are various mechanisms leading to the activation of individual NOS isoforms and thus to NO synthesis. Moreover, NO formed in the reaction catalyzed by different NOS performs various functions:

- NO formed due to bNOS activation: glutamate acts on NMDA receptors of the neuron increasing the level of calcium ions bound with calmodulin. An increase in calcium concentra-

tion activates the bNOS and NO synthesis. In the nervous system NO acts through an increase in the level of cGMP, which activates kinases (positive effect) and through activation of PARS in the presence of O_2^- (adverse effect),

- NO formed due to eNOS activation: the stimulation of muscarine receptors of endothelial cells by acetylcholine, stress, thrombin, ADP, serotonin, bradykin, histamine, norepinephrine, vasopressin, P substance initiates the phosphoinositol cycle, which increases the calcium level, activates eNOS and NO synthesis. NO nitrosylizes proteins producing nitrosothiols, e.g. nitroalbumin, nitrosoglutathione. Nitrosothiols transport NO and act as free NO.
- NO formed due to increased expression of macNOS encoding genes: increased activity of macNOS results from the interaction of macrophages with liposaccharides /LPS/ of themicroorganism cells, TNF alfa, interleukin -1 beta, interferon gamma. The macrophages produce NO; increased NO concentration stimulates the mitochondria to produce O_2^- and H_2O_2. NO combines with O_2^- forming pernitrate ion ($ONOO^-$) which has cytotoxic effects (oxidation of thiols, degradation of carbohydrates, DNA damage, lipid oxidation, inhibition of mitochondrial respiration – death of a cell-antigen) (positive effects). $ONOO^-$ stimulates the production of prostaglandins and leucotrienes in the local arteries - the formation of atherosclerotic plaque (adverse effects)
- NO formed due to the reaction catalyzed by mtNOS: NO acts as an inhibitor of ATP synthesis through:
  - Binding and inhibiting the cytochrome C oxidase (complex IV) competing with oxygen. The cytochrome C oxidase uses 90% of the cell oxygen.
  - Pernitrate produced in the reaction of NO and O_2, which inhibits: ATP synthetase, succinic dehydrogenase, NADH-ubiquinone oxydireductase, aconitase- the Krebs cycle enzyme.
  - S-nitrosylation of 3P-glyceraldehyde dehydrogenase involved in glycolysis.

The activity of NOS is decreased by: phosphorylation, CO and NO association (the NOS product - NO is also its inhibitor - negative feedback). On the other hand, the activity of NOS is increased by tetrahydrobiopterin (BH4) [18].

NO is an unstable, extremely reactive particle involved in many physiological processes, i.e. neurotransmission, regulation of smooth muscle tension, destruction of neoplasms and pathogenic organisms.

NO synthesized by eNOS, also called EDRF [19]:

- regulates the blood pressure - dilates blood vessels
- inhibits the aggregation of thrombocytes
- inhibits the adhesion of leukocytes to the endothelium.

NO synthesized by bNOS:

In the central nervous system, as a neurotransmitter

- regulates the alimentary motor activity
- regulates the local blood flow
- has neuroendocrine functions.

In the central nervous system as a neuromodulator:

- regulates behavioural functions
- affects memory processes - is involved in the production of long-term potentiation (LTP) in the hippocampus, which is indispensable for long-term memory
- increases the response to pain stimuli.

In the autonomous nervous system it:

- inhibits the sympathetic system
- results in oesophageal reflexes during eating.

In combination with O_2^-, it stimulates the nerve cells into dying.

Depending on its concentration, NO synthesized by iNOS:

- has anti-inflammatory effects by producing prostaglandins (low concentration)
- is involved in the immune response and antibacterial, antiviral, antiparasite protection (medium concentration).
- takes part in the pathogenesis of autoimmune diseases, e.g. psorasis, MS, rheumatoid arthritis, systemic lupus erythematosus, ulcerative enteritis (high concentration).

To date several mechanisms of NO action have been detected:

1. Activation of cytosol guanyl cyclase (sGC). sGC is activated by NO through forming with Fe^{+3} the sixth coordinate bond in the sGC haemocycle, which changes the spatial structure of sGC. sGC catalyzes the reaction:

$$GTP \xrightarrow{sGC} cGMP+PPi$$

cGMP formed in this reaction activates phosphodiesterases and kinases as well as cGMP gated channels.

2. S-nitrosylation of proteins. At present S-nitrosylation is thought to regulate many life processes of cells and to be as common as phosphorylation [20]. It consists in the combination of nitroso ion (NO^+) resulting from NO oxida-

tion with the cystein sulphhydro group (-SH), one of 20 amino acids which build proteins. The proteins formed in this way are called S-nitrosothiols and play an important role in various processes [20], i.e. signal transduction, DNA repair, blood pressure control, regulation of ion channels and neurotransmission.

It should be stressed that nitroso cation reacts only with the cystein, which in the amino acid chain is followed by Asp or Glu and is preceded by A (Gly, Ser. Thr, Cys, Tyr, Gln) and B (Lys, Arg, His, Asp, Glu):

AB Cys(Asp or Glu).

3. Activation of poly-(ADP-ribose)-polymerase (PARP). NO in combination with O_2^- forms nitronitrite ($ONOO^-$) which damages DNA resulting in the activation of polyADP-ribose synthetase (PARS) which uses large amounts of NAD. NAD is reproduced from ATP. The cell deprived of ATP dies. This is the way bNOS- synthesized NO acts.

Excessive endogenous NO is involved in the pathogenesis of many diseases, e.g. atherosclerosis, degenerative diseases of the nervous system, inflammations, autoimmune diseases and cancers.

Deficient endogenous NO is implicated in the development of hypertension, pre-eclampsia, arteriosclerosis, hypercholesterolemia [12].

Exogenous NO is administered to patients with venous atherosclerosis of the lower limbs, coronary disease and pregnancy-induced hypertension.

Some reports showed, that NO may be also involved in molecular mechanisms for substances abuse and dependence to opioids, ethanol, and psychostimulants, as cocaine, marihuana and nicotine [21] as well as psychotropic drugs [22]. Moreover, nNOS derived NO participates in the development of rapid tolerance to ethanol and inhibitors of NOS modulate withdrawal from opioids, nicotine and ethanol, diminishing many signs of withdrawal syndrome [23].

Nitric oxide can play role in pathomechanism of alcohol dependence and withdrawal symptoms.

Budzyński et. al [23] in their experiment noticed that in alcohol dependent male patients during 6 months long abstinence period the NO metabolites level was lower than in control group, what may suggest a decrease in NO synthesis or accelerated consumption in this patients group. NO metabolites level in the studied alcoholics was related to intensity of alcohol drinking and severity of their addiction [23].

Their conclusion was that an increase or a slight decrease in NO metabolites plasma concentration following 4-week abstinence may be a predictor of alcohol drinking relapse during the next five months [24].

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