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HOMOCYSTEINE AND HOMOCYSTEINE-RELATED COMPOUNDS: AN OVERVIEW OF THE ROLES IN THE PATHOLOGY OF THE CARDIOVASCULAR AND NERVOUS SYSTEMS

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List of Abbreviations:

5-MTHF - 5-methyltetrahydrofolate

ACE - angiotensin-converting enzyme

ANGII - angiotensin II

BH4 - tetrahydrobiopterin

CBS - cystathionine β-synthase

cGMP - cyclic guanosine 3',5'-monophosphate

CVD - cardiovascular disease

Cys - L-cysteine

eNOS - endothelial nitric oxide synthase

ERK - extracellular-signal-regulated protein kinase

ET-1 - endothelin-1

FAD - flavin adenine dinucleotide

FMN - flavin mononucleotide

GPx - glutathione peroxidase

GSH - reduced glutathione

H_2O_2 - hydrogen peroxide

HO-1 - heme oxygenase 1

iNOS - inducible nitric oxide synthase

IRAK - IL-1R-associated kinase

JNK - Jun kinases/SAPK

MAPK - mitogen-activated protein kinase

MMP - matrix metalloproteinases

MAT - methionine adenosyltransferase

MetRS - methionyl-tRNA synthetase

MS - methionine synthase

MyD88 - myeloid differentiation primary response gene 88

NAC - N-acetyl-L-cysteine

NADPH oxidase - nicotinamide adenine dinucleotide phosphate-oxidase

NF-κB - nuclear factor kappa B

NMDA – N-methyl-D-aspartate

NOS - nitric oxide sinthase

NOS - nitric oxide

O_2^- - superoxide anion

$^{\bullet}OH$ - hydroxyl radical

$ONOO^-$ - peroxynitrite

RBC - red blood cells

SAH - S-adenosylhomocysteine

SOD - superoxide dismutase

TIR - toll/interleukin-1 receptor

TIRAP - toll-Interleukin I receptor domain-containing adaptor protein

TLR4 - toll-like receptor 4

TRAM - TRIF-related adaptor molecule

TRIF - TIR domain-containing adaptor protein inducing interferon-b

TNF-α - tumor necrosis factor alpha

ABSTRACT

Homocysteine, sulfhydryl group containing amino acid, is intermediate product during metabolism of the amino acids methionine and cysteine. Hyperhomocysteinema (HHcy) is used as a predictive risk factor for cardiovascular disorders, the stroke progression, screening for inborn errors of Met metabolism, and as a supplementary test for vitamin B_{12} deficiency. Two organic systems in which homocysteine (Hcy) has the most harmful effects are the cardiovascular and nervous system. The adverse effects of Hcy are achieved by the action of several different mechanisms, such as overactivation of NMDA receptors, activation of TLR-4, disturbance in Ca^{2+} handling, increased activity of NADPH oxidase and subsequent increase of production of reactive oxygen species, increased activity of NOS and NOS uncoupling and consequent impairment in NO and ROS synthesis. Increased production of reactive species during HHcy are related with increased expression of several proinflammatory cytokines, including IL-1β, IL-6, TNF-α, MCP-1, and intracellular adhesion molecule-1. All these mechanisms contribute to the emergence of diseases like atherosclerosis and related complications such as myocardial infarction, stroke, aortic aneurysm, as well as Alzheimer disease and epilepsy. This review provides evidence that supports the causal role for HHcy in the development of CVD and nervous system disorders.

Key words: hyperohomocysteinemia, homocysteine, cardiovascular system, hyperexcitability, nervous system

INTRODUCTION

Homocysteine, sulfhydryl group containing amino acid, is intermediate product during metabolism of the amino acids methionine and cysteine (McBreairty 2016; Selhub and Troen 2016). Homocysteine is nonprotein amino acid which behaves as both a substrate and product of methionine. Homocysteine has key role in methylation cycle, within which a methyl group is transferred to a different substrate (Fernández-Arroyo et al. 2016). Formed homocysteine can be utilized in two ways: 1) homocysteine can be remethylated to methionine by catalytic activity of the enzyme N5, N10-methylenetetrahydrofolate reductase; 2) homocysteine can be converted to cysteine in a reaction that is catalyzed by cystathionine β-synthase (CBS) (Faeh et al. 2006; Ganguly and Alam 2015).

METABOLIC FATE OF HOMOCYSTEINE AND REALTED COMPOUNDS

Methionine is an essential amino acid, whose quantity in the body depends exclusively on the diet. Metabolic importance of methionine is reflected in the large number of transmethylation reactions, which result in transfer of one carbon methyl group to various substrates (DNA, RNA, proteins, phospholipids, polysaccharides, catecholamine, choline) during the methionine cycle. Methionine is also source in synthesis of other sulfur-containing compounds (cysteine and taurine). Taking into account the limitation of dietary supply of methionine, it should be paid attention on importance of methionine synthesis by remethylation of homocysteine (**Figure 1**).

During the methionine cycle, the first step is conversion of methionine to S-adenosylmethionine (SAM), in reaction regulated by ATP and enzyme methionine adenosyltransferase (MAT). Methyl groups can be transferred from SAM to various substrate molecules in reaction catalyzed by various methyltransferases. During these reactions SAM is

transformed into S-adenosylhomocysteine (SAH). SAH is then hydrolyzed to adenosine and homocysteine in reversible reaction regulated enzyme SAH hydrolase. This point has a key role in the further direction of homocysteine metabolism - remethylation or transsulfuration (Ables et al. 2016; Steed and Tyagi 2011).

Homocysteine undergo the remethylation process in case of methionine deficiency. This metabolic pathway requires folic acid as donor of methyl groups for methionine restoration (Pizzolo et al. 2011). Remethylation is catalyzed by methionine synthase (MS), enzyme that uses vitamin B_{12} (cobalamin) as cofactor and 5-methyltetrahydrofolate (5-MTHF) as methyl group donor (**Figure 1**). In this reaction methyl group is transferred from 5-MTHF to homocysteine, resulting in forming new methionine, which can be used for protein synthesis or converted to SAH, again.

Transsulfuration pathway occurs if methionine is present in sufficient amount. The crucial enzyme in this metabolic pathway of homocysteine is cystathionine β-synthase (CBS), enzyme that requires vitamin B_6 as cofactor, and catalyzes reaction of serine and homocysteine to form cystathionine. In next step cystathionine is hydrolyzed by γ-cystathionase (CTH) (also requires vitamin B_6) to cysteine and a-ketobutyrate (Ables et al. 2016; Steed and Tyagi 2011). Some studies showed that exercise can affect the homocysteine metabolism by transsulfuration pathway and decrease homocysteine accumulation and oxidative stress (Deminice et al. 2016).

If there is impairment of remethylation and/or transsulfuration pathway, homocysteine will accumulate in cells, and in these cases of increasing concentrations of homocysteine, it can be converted to more toxic metabolite homocysteine-thiolactone (Jakubowski 2000). Enzyme that catalyzes this reaction in all types of cells is methionyl-tRNA synthetase (MetRS), and this conversion takes place in two phases. The first phase involves the activation of carboxyl group of

homocysteine by ATP and formation of MetRS-bound homocysteinyl adenylate. During the second phase the side chain thiolate displaces the AMP group from the activated carboxyl group of homocysteine, forming homocysteine thiolactone (Jakubowski 1999; Jakubowski 2000).

Homocysteine thiolactone, as a highly reactive compound can acylate amino groups of large number of proteins, forming homocysteinyl groups linked by peptide bonds to proteins, and thus causing the changes in their activity. On the other hand, homocysteine thiolactone can be hydrolyzed by action of calcium dependent enzyme, serum homocysteine thiolactonase, to homocysteine (Jakubowski 2000; McCully 2015).

In human plasma homocysteine is present in various forms, most of it is bound by disulfide bonds to plasma proteins, mainly albumins (around 70%). Approximately 20–30% of plasma homocysteine forms homocysteine dimers or forms dimers with other thiols, and less then 2% is present as free thiol (Hankey and Eikelboom 1999; Refsum et al. 1998). Thus in most of the investigations it was determined total plasma homocysteine, which includes all the above mentioned forms of homocysteine.

BASIC MECHANISMS IN DEVELOPMENT OF HYPERHOMOCYSTEINEMIA

Numerous factors can affect the total plasma homocysteine (tHcy) levels in human plasma, such as gender (woman have lover tHcy than men), nutrition habits (diet deficient in folate, vitamin B_6 and B_{12} leads to increment of tHcy), lifestyle habits (smoking, alcohol consumption, sedentary way of living) (Naik et al. 2007; Nagele et al. 2011; Nilsson et al. 2014; Nygård et al. 1995; Hildebrandt et al. 2015; Jung et al. 2015).

Hyperhomocysteinemia is a condition characterized by increased values of total plasma homocysteine (tHcy) levels in human plasma, above 15 μmol/L (Genest 1999). Depending on the

value of tHcy, hyperhomocysteinemia is classified as mild (tHcy is between 16-30 μmol/L), intermediate (tHcy is between 31-100 μmol/L) and severe (tHcy above 100 μmol/L) (Hankey and Eikelboom 1999). There are also extremely grave forms of hyperhomocysteinemia accompanied by the appearance of homocysteine in the urine (homocysteinuria), when tHcy are even greater than 500 μmol/L (Kumar et al. 2016).

Hyperhomocysteinemia is caused by imbalance in processes and factors involved in the metabolism of homocysteine. Hyperhomocysteinemia can result from four main disorders: 1) genetic abnormalities of enzymes involved in homocysteine metabolism, 2) nutritional deficiencies in folate, vitamin B_6 and vitamin B_{12}, 3) methionine rich diet, and 4) decreased renal function. Two enzymes and three vitamins play a key role in the regulation of circulating homocysteine levels (Nagele et al. 2011; Nilsson et al. 2014; Iacobazzi et al. 2014; Cook and Hess 2005; Perna et al. 2012).

The deficiency in enzymes involved homocysteine metabolism (5,10-methylene tetrahydrofolate reductase (MTHFR), methionine synthase (MS), and cystathionine-β-synthase (CBS)) are rare cause of hyperhomocysteinemia, but can cause the most severe forms of this condition. The most common disorder of enzymes involved in homocysteine metabolism probably is polymorphism of gene coding for the MTHFR (C-to-T substitution at nucleotide 677, and subsequent substitution of Val with Ala), causing the production of thermo labile variant of enzyme (Cortese and Motti 2001). MTHFR catalyzes the reduction of 5,10-methylenetetrahydrofolate (5,10-MTHF) to 5-methyltetrahydrofolate (5-MTHF), in a NADPH-dependent reaction. Although this disorder usually causes mild to moderate hyperhomocysteinemia, the results of recent studies indicate the relationship of MTHFR polymorphism with other diseases (Abd-Elmawla et al. 2016; Jadavji et al. 2015). Other

mutations of MTHFR gene can cause much more severe forms of hyperhomocysteinemia and consequent disorders (Froese et al. 2016). MS plays central role in methionine cycle and folate metabolism. This enzyme catalyze transfer of methyl group from 5-MTHF to homocysteine resulting in regeneration of methionine. Decrement in MS activity will also cause the decrease in SAM content, which acts as methyl group donor for large number of compounds, including DNA, RNA, and proteins. On the other hand decrease of SAM level will increase production of 5-MTHF, whereby MS is only enzyme in mammalian cells that can utilize 5-MTHF, which results in accumulation of 5-MTHF and trapping of folate in this form in cells (Watkins et al. 2002). The most common genetic cause of severe hyperhomocysteinemia is CBS deficiency, which can result in 40-fold increase of tHcy and homocysteinuria in homozygous (Kumar et al. 2016). CBS catalyses the formation of cystathionine from homocysteine and serine during transsulfuration pathway of homocysteine metabolism. Inhibition of CBS activity cause increase of methionine production, and subsequent increase level of SAM. Increased SAM content will decrease activity of MTHFR by feedback mechanism, thereby inhibiting remethilation pathway also (McCully 2015).

Hyperhomocysteinemia can also occur due to dietary insufficient intake of folate (vitamin B_9), vitamin B_{12} and vitamin B_6. These vitamins act as cofactors of enzymes included in homocysteine metabolism, and their blood levels are inversely correlated to tHcy. Because of that many disorders accompanied with hyperhomocysteinemia are treated with B vitamins complex (Kumar et al. 2016). Beside the role in maintaining of the methylation reactions, folate have crucial role in growth and cell division, which is of particular importance during fetal development (Desai et al. 2016). Even in acute application, folic acid exhibit favorable impact on heart and coronary circulation by increasing the outflow of NO, and reducing the production of

free radicals (Djurić et al. 2007). Deficiency of folate in pregnancy leads to neural tube defects and other developmental defects (Blom et al. 2006; Kharb et al. 2016). Additionally occurs mild to moderate hyperhomocysteinemia, which is also associated with a range of disorders. Vitamin B_{12} acts as cofactor for MS, and its deficiency causes impairment of remethylation of homocysteine, hyperhomocysteinemia and stockpiling of 5-MTHF (Hannibal et al. 2016). Vitamin B_6 deficiency is related to impairment of CBS function, considering that act as cofactor of this enzyme (Taysi et al. 2015).

The high methionine intake by diet will cause the increase of tHcy level in plasma considering that half of methionine taken by food is converted to homocysteine (Tyagi 1999; Mandaviya et al. 2014). Thus the excessive dietary intake of groceries rich in methionine (meat, fish) can cause hyperhomocysteinemia. On the other hand, vegetarians can also develop hyperhomocysteinemia due to reduced intake of vitamin B_{12} (Pawlak 2015; Zeuschner et al. 2013).

Kidney is organ that has central role in metabolism of homocysteine, because it contains all metabolizing enzymes: MS, CBS and CTH. Rise in values of tHcy is observed in early stages of renal failure, and during progression of the disease the values of tHcy increase (Ferechide and Radulescu 2009; Amin et al. 2016; Tak et al. 2016). The hyperhomocysteinemia in patients with terminal phases of renal failure (dialysed patients) could be the consequence of several causes: the decreased renal excretion of homocysteine due to impaired renal function, disturbance in homocysteine metabolism, alimentary deficiency in vitamins included in homocysteine metabolism, and undiagnosed genetic abnormalities of metabolizing enzymes (Sette and Almeida Lopes 2014).

Increased tHcy levels can also be increased due to drugs that interfere to metabolic pathways of folate, vitamin B_6 and vitamin B_{12} (Faeh et al. 2006).

ROLE OF HOMOCYSTEINE IN CARDIOVASCULAR PATHOLOGY

Cardiovascular system consists of three components: heart, blood vessels and blood, and participates in maintaining of internal body homeostasis in many aspects: transport of oxygen, carbon dioxide, nutrients, metabolism waste products, and hormones to and from every single cell in the body. The blood vessels are composed of three separated layers: intima, media and adventitia. The intima consists of single layer of endothelial cells that have crucial role in regulation of blood flow. Media is dominantly composed of vascular smooth muscle cells, and represents the thickest layer. The adventitia is outer layer made of connective and fat tissue that have protective role of other inner layers. Blood vessels differ depending on part of circulation to which it belongs (arterial, capillary or venous), or of specific needs and functions of tissues and organs in which it is located. Blood vessels are actively involved in the regulation of blood pressure and blood flow by summarizing the effects of autonomous nervous system, various hormones and endothelial derived factors on changes of diameter. The vascular dilatation caused by shear stress of the blood is mediated by production of endothelium-derived relaxing factor – nitric oxide (NO) (Gutterman et al. 2016). NO is synthesized by NO synthase and diffuses through cell membranes to the vascular smooth muscle cells, increases production of cyclic GMP and induces the relaxation.

Cardiovascular diseases represent the diseases of heart and blood vessels and represent the cause of about one-third of lethal outcomes in the world (Mangge et al. 2014). Since cardiovascular diseases are caused by large number of factors, rarely can be extracted one

particular causative agent of any specific disturbance in functioning of the heart or blood vessels. Increased levels of homocysteine have been associated with a number of vascular complications, and due to this fact hyperhomocysteinemia has been classified as an independent risk factor for atherosclerosis and cardiovascular diseases (Wang et al. 2016; Djuric et al. 2000; Majors et al. 1997; de Jong et al. 1998). Hyperhomocysteinema (HHcy) is used as a predictive risk factor for cardiovascular disorders, the stroke progression, screening for inborn errors of Met metabolism, and as a supplementary test for vitamin B_{12} deficiency (Shoamanesh et al. 2016; Pang et al. 2016; Perry et al. 1995; Zhang et al. 2016*b*; Cioni et al. 2016; Bostom et al. 1999; Folsom et al. 1998). In Framingham Offspring Study homocysteine is stated as one of four factors that increase risk of incident ischemic stroke (Shoamanesh et al. 2016), and earlier studies also showed connection between increased levels of homocysteine and stroke (Pang et al. 2016; Perry et al. 1995). Data from previously conducted studies have shown that elevated levels of homocysteine can be considered as independent risk factor for coronary heart disease (Zhang et al. 2016; Cioni et al. 2016; Bostom et al. 1999; Folsom et al. 1998).

Almost fifty years ago Kilmer McCully described the case of child with elevated concentrations of homocysteine, cystathione and homocysteine-cysteine disulfide in plasma and urine and low levels of methionine in plasma (McCully 1969). During necropsy author found many lesions composed of loose fibrous connective tissue in medium-sized and small arteries of many tissues and organs, due to increased homocysteine levels, what was the basis for the homocysteine theory of atherosclerosis. Since then, many investigations dealing with the effects of homocysteine on cardiovascular tissues and roles of homocysteine in pathogenesis of numerous cardiovascular disorders. Experimentally and clinically data have shown a variety of adverse effects of homocysteine including impaired endothelium-dependent relaxation regulated

by nitric oxide and endothelium-derived hyperpolarizing factor, proliferation of vascular smooth muscle cells, increment and oxidation of low-density lipoprotein, decrease of thrombomodulin expression (Cheng et al. 2011; Zhang et al. 2016*a*; Glueck et al. 2016; Yang et al. 2016).

Due to innately lower amount of CBS enzyme in cardiomyocytes and cell types represented in vascular tissues could be more sensitive to homocysteine toxicity (Chen et al. 1999; Tyagi et al. 2009). There is no unique, comprehensive theory which includes all effects of Hcy on cardiovascular system, considering that this compound damages several cell types through multiple mechanisms (**Figure 2**). Furthermore, taking into account results of investigations that showed that Hcy-lowering therapy have not shown clinical efficacy there are not enough facts to support routine screening and treatment of elevated Hcy levels (Djuric et al. 2008; Martí-Carvajal et al. 2017).

Taking into account large number of critically important functions of endothelial cells: platelet adhesion and coagulation, regulation of cellular growth, maintenance of vasomotor function and immune function, endothelial dysfunction has been referred as key pathological condition in generation of cardiovascular diseases (Goldenberg and Kuebler 2015; Knolle and Wohlleber 2016). Endothelial dysfunction can be defined as disruption of homeostasis between vasodilatation and vasoconstriction.

One of the most frequently mentioned mechanisms that link Hcy to endothelial dysfunction is oxidative stress (Tyagi N et al. 2005) (**Table 1**). Homocysteine induces increase in production of reactive oxygen and nitrogen species (ROS/RNS), and this increment in content of these highly reactive molecules is closely associated with endothelial dysfunction and other cell species in the vascular wall (Mangge et al. 2014). The major source of ROS in the cell is mitochondrial respiration, but under physiological conditions there is balance in production and

degradation of free radicals. Some other enzymes also contribute in ROS generation, like nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase), nitric oxide synthase (NOS), lipoxygenases, cytochrome P-450. Hyperhomocysteinemia is associated with production of different ROS: superoxide anion (O_2^-), hydroxyl radical ($^{\bullet}OH$), peroxynitrite ($ONOO^-$), hydrogen peroxide (H_2O_2), as well as other peroxides and hypochlorous acid, and their organic analogues (Papatheodorou and Weiss 2007). Within the investigation where the coronary and mesenteric arteries were incubated with methionine it has been revealed the role of angiotensin-converting enzyme (ACE) and angiotensin II (ANGII) signaling pathway in activation of NADPH oxidase and increase in O_2^- production (Huang et al. 2015). The link between ACE, ANGII signaling and NADPH oxidase have been noticed earlier, but in this study it was described the role of homocysteine in activation of ACE (Huang et al. 2012). Namely, homocysteine induces homocysteinylation of ACE, which in turn have greater activity of this enzyme, followed by increased transduction by ACE/ANG II/AT_1R signaling pathway, and increased activation of NADPH oxidase and consequent production of O_2^-.

On the other hand homocysteine induces increased expression of different forms of NADPH oxidase (**Table 1**). NOX4 is isoform of NADPH oxidase highly represented in the kidney, and incubation of tubular cells with homocysteine showed increased production of O_2^- (Hwang et al. 2011). Proposed underlying mechanism was increment expression of NOX4 by homocysteine. The above mentioned changes induced by homocysteine were abolished by supplementation of folic acid, and consequent decrease of homocysteine level in hyperhomocysteinemic rats. Murray and colleagues indicated to another link between NOX4 and homocysteine metabolism (Murray et al. 2015). Results of their study showed that activity of NOX2 and NOX4 derived reactive species have pivotal role in balancing between remethylation

and transsulfuration pathways. In NOX4 deficient mice directing homocysteine through transsulfuration pathway was reduced, as well as amount of synthesized cisteine, which is necessary for the synthesis of glutathione, a basic endogenous antioxidant (Figure 1). Namely, half of the required amount of cisteine is provided from homocysteine through transsulfuration pathway, and therefore, significant reduction of cisteine production from homocysteine has considerable metabolic consequences (Mosharov et al. 2000). Furthermore, Smith and coauthors shoved that inhibition of NOX4 worsens the dilation induced by acetylcholine in blood vessels previously exposed to homocysteine thiolactone (Smith et al. 2015). Different NADPH oxidase isoform, NOX2 is the mostly represented in the endothelium. Incubation of Human Umbilical Vein Endothelial Cells (HUVEC) with homocysteine induced significant increase in NOX2 protein expression, as well as increase in nuclear localization of $p47^{phox}$ (Sipkens et al. 2013). These changes induced by homocysteine were correlated with increased production of O_2^-, accumulation of nitrotyrosine residues and apoptosis of endothelial cells. Similar changes were detected in cardiomyocytes (Sipkens et al. 2011). Based on these facts, it can be concluded that homocysteine increases the expression of different isoforms of NADPH oxidase, as well as activation this enzyme, resulting in both cases in increase of O_2^- content.

Increased production of O_2^- leads to decreased bioavailability of nitric oxide (NO), due to reaction of these two molecules and production of highly reactive peroxynitrite ($ONOO^-$). NO is synthesized by three isoforms of enzyme nitric oxide synthase (NOS): endothelial NOS (eNOS or NOSI), inducible NOS (iNOS or NOSII) and neuronal NOS (nNOS or NOSIII). eNOS and nNOS are constitutive enzymes and their activity is regulated by changes in Ca^{2+} content in the cytoplasm. All three isoforms generate NO from L-arginine in the presence of O_2 and NADPH. Also as cofactors are necessary flavin mononucleotide (FMN), flavin adenine dinucleotide

(FAD), and tetrahydrobiopterin (BH4). BH4 is crucial for NOS function because it binds NOS monomers to form dimers which contain two reductase domains 'coupled' to another pair of oxygen domains (Lee et al. 2016). NOS monomers generate O_2^- instead of NO, and this 'uncoupled' enzyme represents a ROS producer. The classical signaling pathway of NO includes activation of soluble guanylyl cyclase and production of cyclic guanosine 3',5'-monophosphate (cGMP). NO acts on autocrine or paracrine manner. In study on HUVECs increased homocysteine induced decrement in NO production, and also increment in production of endothelin-1 (ET-1), as one of the most potent vasoconstrictor (Tian et al. 2016). On the other hand homocysteine can increase production of NO by upregulation of iNOS by proinflammatory cytokines, which synthesis is amplified by homocysteine. But in these conditions homocysteine induces increased expression of iNOS, and increment of O_2^- production by uncoupling of iNOS (Duan et al. 2006).

Recently, it has been indicated potentially new mechanism involved in Hcy induced changes in cardiovascular system (CVS) which implies Toll-like receptor 4 (TLR4) (**Table 1**) (**Figure 3**). TLR4 belongs to the Toll-like receptor (TLR) family, and their roles in pathogenesis of CVD are intensively studied. Namely, TLR4, that normally play a role in the innate immune response and recognize viral or bacterial antigen motifs, are expressed in almost all cells represented in CVS (Becher et al. 2018) and participate in pathogenesis of atherosclerosis (den Dekker et al. 2010), ischemic heart disease (Satoh et al. 2016), heart failure (Liu et al. 2015) or aorta aneurism (Lai et al. 2016). Results of Jeremic and colleagues indicates that ablation of TLR4 in HHcy mice diminish changes induced by increased levels of Hcy such as left ventricular hypertrophy, increased oxidative stress and decreased antioxidative capacity, and mitochondrial fission (Jeremic et al. 2017*a*). Same authors provided results on the basis of which

it can be concluded that TLR4 during HHcy mediate in predominance of mitochondrial fission in endothelial cells, and consequent oxidative stress, endothelial cell loss and dysfunction, increased collagen deposition, which ultimately causes hypertension (Jeremic et al. 2017*b*). On the other hand, mutation of TLR4 alleviated vascular inflammation and prevented hypertension (Familtseva et al. 2016). Molecular mechanisms and signaling pathways involved in above mentioned changes in CVS induced by HHcy trough TLR4 are probably same or similar to those that exist in other cells and tissues. Intracellular domain of TLR4, Toll/interleukin-1 receptor (TIR) domain, activates several adopter proteins such as: myeloid differentiation primary response gene 88 (MyD88), Toll-Interleukin I receptor domain-containing adaptor protein (TIRAP), TIR domain-containing adaptor protein inducing interferon-b (TRIF), TRIF-related adaptor molecule (TRAM), which actually represents the first step of TLR signal transduction (Goulopoulou et al. 2016). MyD88 further engages IL-1R-associated kinases (IRAKs) which initiate activation of nuclear factor kappa light-chain-enhancer of activated B cells (NF-κB) and production of cytokines. Another, TRIF pathway, requires TRAM, which through activation of several kinases (receptor-interacting serine/threonine-protein 1 (RIP-1) kinase and transforming growth-factor-b-activated kinase 1 (TAK-1)) activates NF-κB and mitogen-activated protein kinase (MAPK), and overall result is increment of expression of proinflammatory genes and synthesis of inflammatory cytokines (**Figure 3**) (De Nardo. 2015; Li et al. 2014).

Effects of Hcy and its different forms on heart, coronary circulation and heart tissue oxidative balance were intensively investigated by Jakovljevic and Djuric. In study dealing with correlation between plasma total Hcy (tHcy) level and coronary atherosclerosis, it has been shown that tHcy was significantly higher in patients with angiographically conformed coronary artery disease (CAD) compared to healthy control (Mitrovic et al. 2002). Besides that, in group

of patients with CAD level of tHcy more frequently exceeded the value of 15 mmol/l, which was also more common with older people, and there was positive correlation between increased tHcy level and uric acid level. In study that examined the effects of various Hcy-related compounds (DL-Hcy, DL-Hcy thiolactone-hydrochloride (TLHC) and L-Hcy TLHC) the authors emphasized their harmful effects on cardiac function during acute administration in isolated rat heart (Zivkovic et al. 2012). During acute application of DL-Hcy TLHC simultaneous inhibition of production of the gasotransmitters (NO, H_2S and CO) additionally exacerbated the effects of DL-Hcy TLHC (Zivkovic et al. 2013). The inhibition of CO production expressed most deleterious effects in comparison to deprivation of NO and H_2S production. Furthermore, results of another investigation showed protective effects of H_2S in changes induced by DL-Hcy (Stojanovic et al. 2016*a*). Namely, application of DL-propargylglycine, as an inhibitor of H_2S formation, decreased all cardiodynamic parameters and increased the concentration of O_2^-, which was even more pronounced with the simultaneous application with DL-Hcy, which leads to the conclusion that DL-Hcy shows a lower pro-oxidative effect in the presence of H_2S. It is also shown that previously mentioned Hcy-related compounds (DL-Hcy, DL-Hcy TLHC and L-Hcy TLHC) impair oxygen consumption of rat heart tissue homogenate, as well as the inclusion of the gasotransmitters NO, H_2S and CO in these effects (Uzelac et al. 2017).

Results of several investigations even few decades ago indicated the link between Hcy and N-methyl-D-aspartate (NMDA) receptors (**Table 1**). NMDA receptor belongs to the heterogeneous family of ionotropic glutamate receptors, whose roles are best known within the function of the nervous system, but also in has been shown that functional NMDA receptors are expressed in a variety of non-neuronal cells, among others in the cells of the cardiovascular system (Gill et al. 1988; Chen et al. 2005; Doronzo et al. 2010). Functional NMDA receptors are

heterotetramers that contain two obligatory GluN1 subunits and two more GluN2 and/or GluN3 subunits (Vyklicky et al. 2014; McGee and Abdel-Rahman. 2016). However, Western Blot analysis in the work of Leung and colleagues have shown that GluN2 subunits are not present in adult rat heart, while GluN1 subunits are expressed in the atrium and ventricle, which caused speculation that the NMDA receptors at the heart could be composed of homooligomeric GluN1 subunits (Leung et al. 2002). On the other hand other study showed transient expression of GluN2B subunit in perinatal cardiac myocytes (Seeber et al. 2004), while previous research have indicated the presence of GluN2C subunit in the heart (Lin et al. 1996). NMDA receptors in the heart have important role regulation of electrical activity of conductive system of the heart, and therefore could play a role in arrhythmogenesis (D'Amico et al. 1999; Shi et al. 2014). Hcy, as NMDA receptor activator, induced shortening of SA nodal recovery time and AV nodal effective refractoriness, causing proarrhythmogenic condition, while Mg^{2+}, as endogenous inhibitor of NMDA receptors mitigated changes induced by HHcy (Soni et al. 2016). Several studies confirmed anti-arrhythmic effects of NMDA receptor blockers, such as MK-801. NMDA receptor inhibition reduced the likelihood of arrhythmias in conditions of ischemia and reperfusion (Sun et al. 2014) and increased heart rate variability, which also has anti-arrhythmic properties (Shi et al. 2017). It is also shown that NMDA receptors, at least partially, participate in changes of heart function induced by Hcy (Rosenberger et al. 2006) (**Figure 3**). In fact, most of the studies dealing with effects of the NMDA receptors modulation on the cardiac function actually examined the influence of NMDA receptors in different parts of CNS on heart. Aim of research by Srejovic and colleagues was to determine the role of NMDA receptors in cardiac function, as well as the possible role played by the oxidative stress induced by the overstimulation of NMDA receptors in isolated rat heart induced by acute application of DL-Hcy

TLHC (Srejovic et al. 2015). Authors combined application of DL-Hcy TLHC and MK-801 (NMDA receptor blocker) and based on results of this research it is clear that NMDA receptors in heart do have role in regulation of cardiac function, as well as that Hcy achieve its effect through NMDA receptors. Similar results were achieved by another NMDA receptor antagonist, memantine, and ifenprodil, as negative modulator of NMDA receptor function (Srejovic et al. 2017). There are several proposed mechanisms and signaling paths that mediate in effects induced by NMDA receptor activation. One of the most important features of NMDA receptors, which make them unique in relation to other ionotropic glutamate receptors, are high conductivity for Ca^{2+} (Vyklicky et al. 2014) (**Figure 3**). Increased Ca^{2+} inflow and overload due to activation of NMDA receptors induces disruption of Ca^{2+} homeostasis and consequent ROS production, disturbances in mitochondrial function and pro-apoptotic environment in the cell (Gao et al. 2007). Increased Ca^{2+} influx activates phosphoinositide 3-kinase and protein kinase B (PI3K-Akt signaling pathway), which further induces phosphorylation and consequent activation of protein kinase C (PKC), NOX and mitogen-activated protein kinase (MAPK): 1) extracellular-signal-regulated protein kinases (ERKs), 2) Jun kinases/SAPK (JNKs) and 3) p38 (McGee and Abdel-Rahman. 2014; McGee and Abdel-Rahman. 2016). Increased NOX activity induces increased production of ROS. Increment of ROS production additionally activates MAPK, which further activates NF-κB and proinflamatory environment (Pang et al. 2014). Furthermore, Ca^{2+} overload and activation of PI3K induce increased production of NO by NOS (Simon et al. 2014). But, as indicated above, NO in conditions of increased synthesis of O_2^-, forms $ONOO^-$, while pro-inflammatory cytokines induces increased expression of iNOS, uncoupling of NOS and increased production of O_2^- by NOS, thus creating a vicious circle (Mangge et al. 2014, Campos-Mota et al. 2017).

Bearing in mind that inflammation is important factor in pathogenesis of cardiovascular diseases, a certain number of papers dealt with role of Hcy in induction of inflammation and showed that HHcy is accompanied with upregulation of several pro-inflammatory cytokines, including IL-1β, IL-6, TNF-α, MCP-1, and intracellular adhesion molecule-1 (**Table 1**). NMDA receptors are also present in almost all blood cells so that their activation may have various effects on the signaling pathways in these cells. Activation of NMDA receptors in red blood cells (RBC) induces increase in Ca^{2+} content and thus changes properties of RBCs such as cell volume, membrane steadiness, and capacity to transfer O_2 (Makhro et al. 2017; Makhro et al. 2013). On the other hand, Reinhart and coworkers indicated that neither activation nor inhibition has any influence biophysical properties of RBCs, such as deformability and aggregability parameters (Reinhart et al. 2011). Namely, treatment of RBCs with homocysteic acid, and combination of memantine and homocysteic acid did not change any of observed parameters. Bearing in mind that plasma concentrations of Hcy in healthy population are below 15 μmol/l, in cases of severe HHcy, when Hcy concentration exceeds 100 μmol/l, homocysteine and homocysteic acid probably have a dominant role in the regulation of NMDA receptor activity in the blood cells (Adragna 2010). The presence of the NMDA receptors in several types of immune competent cells was also confirmed, suggesting their role in regulation of immune response and inflammation (Boldyrev et al. 2012). Similar to the aforementioned mechanisms in other types of cells, activation of NMDA receptors by homocysteine or homocysteic acid in cells of immune system induces Ca^{2+} accumulation, and consequent increase of reactive species production and oxidative stress, and activation of MAPK (Boldyrev et al. 2013). Activation of neutrophils causes increased expression of NMDA receptors on their membranes, so in the conditions of HHcy neutrophils generates large amount of ROS and easier undergo to

degranulation process (Bryushkova et al. 2011). These changes produce pro-inflammatory environment and enhance the production of pro-inflammatory cytokines such as TNF-α, interleukin-1β and interleukin-6 in neutrophils, monocytes or macrophages via NF-κB, ERK, and P2X7 stimulation (da Cunha et al. 2010; Zanin et al. 2015). Augmented production of pro-inflammatory cytokines and activation of immune cells activates both necrotic and apoptotic cell death. Platelets also express NMDA receptors, and results of several studies suggest their significant role in the functioning of platelets. NMDA receptor antagonists, such as MK-801 and memantine, induce inhibition on platelet aggregation and activation, while NMDA receptor agonists increased aggregation in the presence of low concentrations ADP with an increase in Ca^{2+} content in platelets (Kalev-Zylinska et al. 2014). In accordance with the above fact, it has been shown that Hcy causes an increase in whole blood platelet aggregation, as well as in production of O_2^- (Karolczak et al. 2017). Antagonists of NMDA receptors induced reduced increment of platelet aggregation induced by Hcy, while increased production of O_2^- remained unchanged, suggesting other possible mechanism involved, independent of Ca^{2+} entry.

Activation of ERK and Akt by Hcy induces increment of expression of matrix metalloproteinases (MMP) in macrophages (Lee et al. 2012) (**Figure 3**) (**Table 1**). Activity of MMPs lead to destruction of extracellular matrix which may result in a rupture of the atherosclerotic plaque. It has also been shown that it is in patients ascending aortic aneurysms there is positive correlation between serum level of Hcy and activity of MMP-3 and MMP-9 (Tsarouhas et al. 2011; Vacek et al. 2012).

Literature data suggest that homocysteine might predispose to cancer is the activation of pro-inflammatory genes due to region-specific hypomethylation. Results of *in vitro* and *in vivo*

experiments have suggested that homocysteine might provoke intestinal mucosal injury by modulating TNF-α-mediated cytotoxicity (Oussalah et al. 2011; McCully 2009).

Martí-Carvajal and colleagues presented updated review regarding homocysteine-lowering interventions and their effects on prevention of cardiovascular events, as well as reduction of all-cause mortality (Martí-Carvajal et al, 2017). Authors of this third update of the Cochrane review shoved that there were no differences in effects of homocysteine-lowering interventions in the form of supplements of vitamins B_6, B_9 or B_{12} given alone or in combination comparing with placebo on myocardial infarction, death from any cause or adverse events. In terms of stroke, this review found a small difference in effect favoring to homocysteine-lowering interventions in the form of supplements of vitamins B_6, B_9 or B_{12} given alone or in combination comparing with placebo. Authors suggested there was a need for additional trials that would be more comprehensive and in which the effects of antihypertensive therapy only should be compared with combined therapy by antihypertensive drugs and homocysteine-lowering maneuvers, as well as the effects of different doses of homocysteine-lowering substances.

HOMOCYSTEINE AND RELATED COMPOUNDS, NERVOUS SYSTEM AND HYPEREXCITABILITY

In addition to the previously mentioned fact that Hcy represent independent risk factor for cardiovascular diseases, there is a growing interest in examining the role of Hcy in the pathogenesis of neurological disorders (**Table 2**). For instance, HHcy is brought into the connection to cognitive decline, it has been practically demonstrated that there is a positive correlation between increased values of Hcy and reduction of cognitive functions (Setién-Suero et al. 2016). One of the most studied form of cognitive impairment certainly is Alzheimer's

disease, where it has been shown that Hcy is one of the risk factors (Janel et al. 2017; Moretti et al. 2017). Some authors indicate the correlation between pathophysiological changes in the vascular system and nervous disorders. In study by Moretti and colleagues it was shown that low vitamin D concentrations and HHcy are common characteristics in two types of dementia, subcortical vascular and Alzheimer (Moretti et al. 2017). There are probably similar pathogenetic mechanisms by which Hcy induces changes in the cardiovascular and nervous system. Longoni and coworkers analyzed changes in glial reactivity in rat astrocyte cultures induced by Hcy (Longoni et al. 2017). Results of this study showed that Hcy induced decrease in the activities of Na^+, K^+ ATPase, superoxide dismutase (SOD), and glutathione peroxidase (GPx), as well as in the reduced glutathione (GSH) content. On the other hand Hcy induced increase in transcription for nuclear factor kappa B (NFκB) and decrease in expression of heme oxygenase 1 (HO-1). Similarly to the abovementioned changes induced by Hcy in cardiovascular system, there is analogous pattern in nervous tissues. Namely, Hcy induces disruption of blood-brain barrier trough NMDA receptor overstimulation and consequent excitotoxicity (Kamat et al. 2016). Activation of MMPs, due to disturbed Ca^{2+} signaling during HHcy, further disrupts tight junctions alleviating damage of blood-brain barrier. Administration of MK-801 and H_2S mitigates changes induced by Hcy (Kamat et al. 2013). Furthermore, Hcy induces inflammation in nervous tissue via NF-κB and increases pro-inflammatory cytokines, (TNF-α, IL-1β and IL-6), chemokine and prostaglandin E (da Cunha et al. 2010; da Cunha et al. 2012).

A group of researchers gathered around Djuric and Stanojlovic carried out a large number of studies dealing with investigation of the role of Hcy in the pathophysiology of epilepsy and seizures. Stanojlovic and colleagues examined effects of increasing doses of DL-homocysteine thiolactone (DL-Hcy TLHC) on brain electrical activity (Stanojlović et al. 2009).

Electroencephalographic (EEG) recordings showed the two types of seizures, the coexistence of convulsive and nonconvulsive epilepsy. The results of this investigation indicated that acute administration of DL-Hcy TLHC significantly changes neuronal activity, EEG tracings, and behavioral responses. The same research group examined the effect of ethanol on changes in the nervous activity induced by DL-Hcy TLHC (Rasić-Marković et al. 2009*a*). Results indicated that ethanol alone increased EEG spectral power density with a marked spectrum shift toward low frequency waves. On the other hand, highest applied dose of ethanol in combination with DL-Hcy TLHC, actually decreased EEG spectral power density, while lower doses had opposite effects. These data depict complex scheme of influence of ethanol on CNS functions and interrelationship of ethanol and epilepsy. The aim of next investigation was to assess the effects of MK-801, as noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist, and ifenprodil, as negative modulator of NMDA receptors, in order to elucidate role of NMDA receptors in Hcy-induced seizures (Rašić-Marković et al. 2011). Administration of MK-801 30 minutes prior to DL-Hcy TLHC significantly decreased number of seizure episodes, and also showed tendency to reduce incidence of convulsions, latency to the first seizure onset and the severity of seizure. Ifenprodil had different effect, mitigation of latency to the first seizure onset and increment of number of seizure episodes. These results indicate that MK-801 has potentially anticonvulsive effect and that NMDA receptors perhaps could be the target for seizures reduction. In study by same experimental group conducted by Hrncic and coauthors it was assessed the relationship between sleep deprivation and epilepsy (Hrncić et al. 2013). Selective deprivation of paradoxical sleep in adult rats induced changes in EEG and behavior which can be characterized as factors that could facilitate Hcy-induced seizures in rats. On the other hand, results of another study pointed out beneficial impact of physical activity on Hcy-induced

seizures in rats (Hrncic et al. 2014*a*). Experimental group of rats that which were physically active expressed increased seizure latency and decreased number of seizures, compared to sedentary rats treated with Hcy. In the same time, it has been shown that exercise induces increment of antioxidant capacity which can be, at least partially, the cause of the positive effect of physical activity on epileptogenesis. The subject of the next study was the assessment of relationship between Hcy, oxidative stress and behavioral changes (Hrncic et al. 2016). HHcy in rats was induced by methionine-enriched diet and oxidative stress was altered in different regions of brain. HHcy induced anxiety was associated with simultaneous increase of index of lipid peroxidation in the cortex and caudate nucleus, which leads to the conclusion that proanxiogenic effects of HHcy could be consequence of oxidative stress in the rat brain.

The importance of vitamin B complex (folate, B_6 and B_{12}) in Hcy is well known (**Figure 1**) so there is a question about the effects of vitamin B supplementation on the epileptogenic potential of Hcy. Results of study aimed to compare the effects of co-administration with folic acid (vitamin B_9) and Hcy and Hcy alone on seizures incidence, median number of seizure episodes and severity in adult rats showed no difference in these two groups, but activity of Na^+, K^+ ATPase and Mg^{2+} ATPase was significantly increased (Rasic-Markovic et al. 2015). It has been previously proven that DL-Hcy TLHC strongly inhibits activity of Na^+, K^+ ATPase in various parts of the brain, such as hippocampus, cortex and brain stem of rats (Rasić-Marković et al. 2009*b*). This blockade of Na^+, K^+ ATPase in mentioned brain structures certainly play important role in convulsive and excitotoxic features of Hcy. In study where folic acid was applied with L-arginine in subchronic fashion, there was a tendency to increase latency and decrease the number of seizure episodes in Hcy treated rats (Rasic-Markovic et al. 2016). Based on this data it can be concluded that folic acid can exhibit some anticonvulsive effects.

Previously published study confirms this assertion (Marković et al. 2011). Namely, administration of folic acid 30 minutes prior to DL-Hcy TLHC decreased the incidence of seizures and increased latency, as well as mean total spectral power density in EEG recording. According to results of other studies where supplementation with folic acid alone did not produce desired results, namely plasma levels of Hcy were decreased, but cellular levels were unchanged, arises conclusion that it would be rationale to investigate effects of group of metabolically interconnected vitamins to pathological changes caused by Hcy (Smith et al .2013, Mei et al. 2010). Although vitamins B_6, B_{12} and folic cycle first foray into focus due to Hcy metabolism, it is crucial to emphasize that the other B vitamins have important role in Hcy metabolism, niacin (B_3) as cofactor for the enzymes in the folate and methionine cycles (dihydrofolate reductase and S-adenosylhomocysteine synthase), and riboflavin (B_2) as cofactor for methylenetetrahydrofolate reductase (MTHFR) and methionine synthase reductase (MTRR) (McCormick 2007; Marashly and Bohlega 2017; Kennedy 2016). Morris and coworkers showed that folic acid have protective impact on cognitive function only if level of vitamin B_{12} was within physiological range; if vitamin B_{12} was deficient high folate status potentiated detrimental effects of vitamin B_{12} deficiency (Morris et al. 2007).

In order to further dissect the mechanism of deleterious effect of Hcy in brain function Djuric and Stanojlovic research group also explored the role of gasotransmitters, primarily nitric oxide (NO) (Hrnčić et al. 2012; Stojanović et al. 2015). In the research regarding the effect of L-arginine, as NO precursor, and L-NAME, as nitric oxide synthase (NOS) inhibitor, it has been shown that L-arginine, in a dose-dependent manner, decreases lethality, seizure incidence and the number of seizure episodes and increases latency time to the first seizure induced by DL-Hcy TLHC, while, on the other hand L-NAME have had quite opposite effects (Hrncić et al. 2010).

The results of this study further clarified the epileptogenic effects of Hcy and pointed to the anticonvulsant significance of NO. In next study authors assessed effects of 7-nitroindazole, as selective inhibitor of neuronal NOS (nNOS), in Hcy induced seizures and singled out nNOS as a key enzyme in protective effects of NO (Hrncić et al. 2012*a*). Namely, 7-nitroindazole increased the seizure incidence and the number of seizure episodes per rat, as well as severity of Hcy-induced seizures. Beside the nNOS, inducible form of NOS (iNOS) also has important role in brain NO signaling. Block of iNOS by aminoguanidine shortened seizure latency time and augmented the number and severity of seizures induced by Hcy (Hrnčić et al. 2014*b*). Aminoguanidine also potentiated the proepileptogenic changes in EEG recording, such as number and duration of spike and wave discharges. Besides that, Hcy also showed propensity to alter the activity of acetylcholinesterase activity in rats with HHcy (Hrnčić et al. 2014*c*). In that manner Hcy could be considered as neuromodulator, bearing in mind its indisputable hyperexcitability properties (Hrncic et al. 2018; Hrncic et al. 2014*d*).

OTHER SULFUR CONTAINIG COMPOUNDS: PURPOSES AND ROLES

Beside Hcy and its various metabolites there are many other sulfur-containing compounds with the most diverse roles in the body. The substances of the highest significance that contain sulfur are amino acids methionine, cysteine, and taurine. Methionine and cysteine are proteogenic amino acids involved in structure of various proteins who often have crucial roles in physiological processes (Djuric 2018). Derivatives of sulfur-containing amino acids, glutathione and N-acetylcysteine, represent powerful intrinsic antioxidant agents which are continuously involved in the neutralization of free radicals (Colovic et al. 2018). Furthermore, sulfur-containing amino acids have chelating site for heavy metals and because of that have beneficial

effects in eliminating toxic metals. In that sense, their application with chemotherapeutic drugs such as cisplatin seems quite rational. Although cisplatin is irreplaceable and the drug of first choice in many malignances, cisplatin therapy is accompanied large number of side effects, such as nephrological, hepatological, gastrointestinal, reproductive, hematological, cardiological, etc. Almost all of mentioned side effects are consequence of oxidative damage induced by cisplatin in various tissues. Taking into account information available so far, sulfur-containing amino acids could be used as protective supplementation in prevention adverse effects of metal-containing antineoplastic drugs because they do not interfere with antitumor properties, are not toxic unless they are taken in excessive amounts, and have strong antioxidative potential (Rosic et al. 2018). L-cysteine (Cys) and N-acetyl-L-cysteine (NAC) exhibit protective role against deleterious effect of Hcy, although they all contain sulfur. For instance, colon damage induced by high intake of methionine and consequent HHcy, could be prevented with Cys and NAC (Stojanović et al. 2018*a*). Quite similar effects were achieved in liver tissue, where Cys and NAC improved antioxidative defense by increasing the activity of antioxidant enzymes and decreased tissue damage induced by subchronic methionine exposure (Stojanović et al. 2018*b*). On the other hand, HHcy induced by methionine as well as DL-Hcy TLHC in acute administration had opposite effect in mentioned tissues (Stojanović et al. 2016*b*, Stojanović et al. 2017).

CONCLUSION

As an integral component of several disorders including cardiovascular and cerebrovascular diseases, neurodegeneration, liver steatosis, hyperhomocysteinemia can result from deficiencies of vitamin cofactors (B_6, B_{12}, folic acid) required for Hcy metabolism and/or from genetic disorders of its metabolism. This review provides evidence that supports the causal role for HHcy in the development of CVD and nervous system disorders, and outlines several cellular and molecular mechanisms by which Hcy induces disorders of the function of these two organic systems. These mechanisms include oxidative stress, inflammation, ER stress, DNA hypomethylation, homocysteinylation, Hcy thiolactone levels and mitochondrial dysfunction. In the future, further studies are much needed to provide more convincing evidence demonstrating the pathogenic role of HHcy in the progression of CVD and nervous system disorders in animal studies and clinical studies. Beside mentioned facts, it remains to be examined whether Hcy is a causative agent or marker of damage.

CONFLICTS OF INTEREST

No conflicts of interest, financial or otherwise, are declared by the author(s).

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Table 1. **Mechanisms mediating the harmful effects of homoscysteine in cardiovascular system**.

Proposed mechanism	Relevant papers
Activation of Toll-like receptor 4	Jeremic N et al. 2017*a*; Jeremic N et al. 2017*b*.
Overactivation of NMDA receptors	Gao et al. 2007.
Induction of oxidative stress	Tyagi N et al. 2005.
Upregulation of NADPH oxidase and increase in O_2^- production	Huang et al. 2015; Liu et al. 2015.
Activation of ACE and increase in production of ANGII	Huang et al. 2012.
Disturbed NO generation	Topal et al. 2004.
Induction of inflammation	Boldyrev et al. 2013; Zanin et al. 2015; Bryushkova et al. 2011.
Increment of expression of MMP	Lee et al. 2012; Tsarouhas et al. 2011; Vacek et al. 2012.
Impairments of the conduction system of the heart function	Soni et al. 2016; Sun et al. 2014.

Table 2. **Mechanisms mediating the harmful effects of homoscysteine in nervous system**.

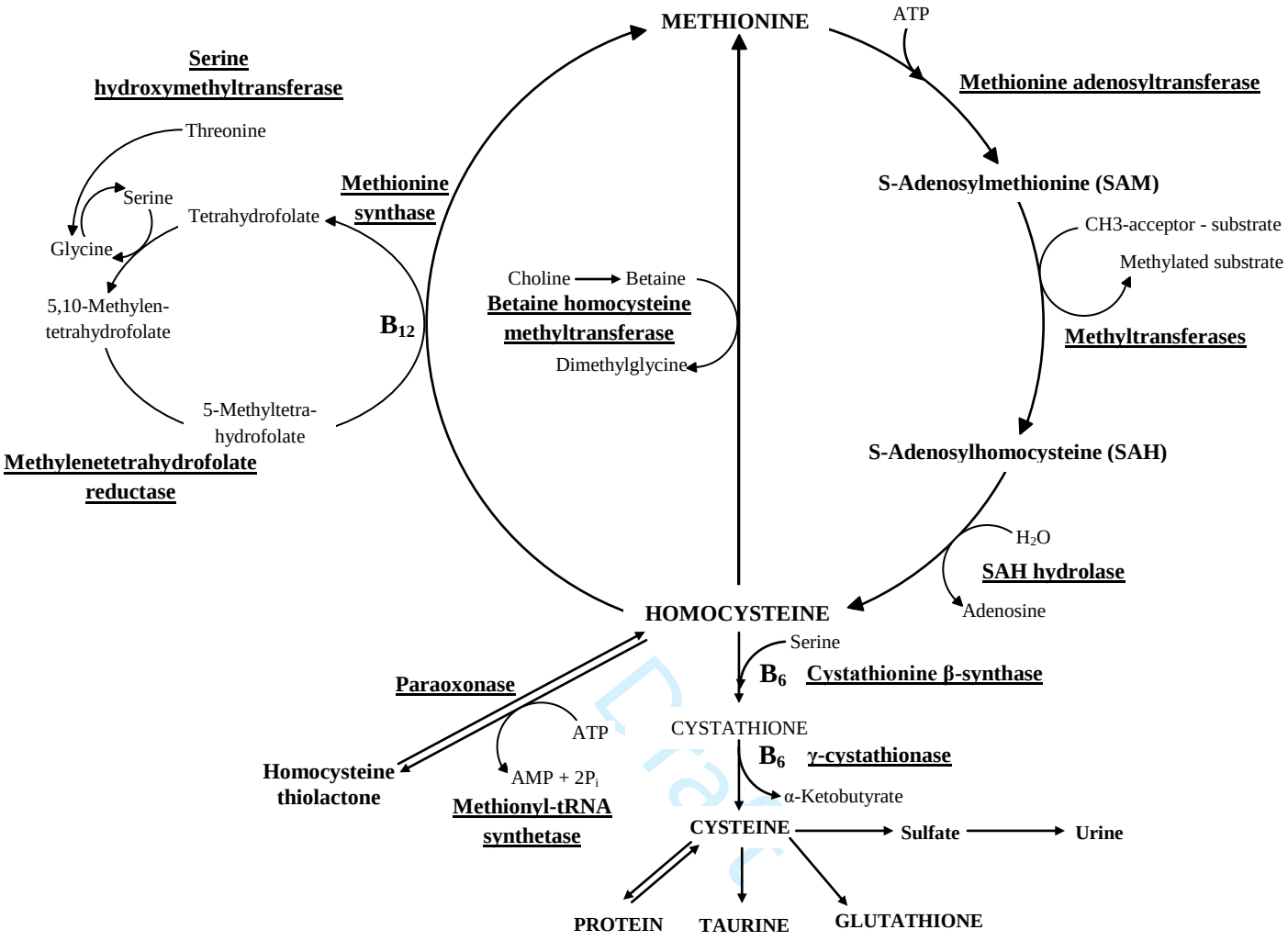
Proposed mechanism	**Relevant papers**
Decrease of Na^+, K^+ ATPase activity	Longoni et al. 2017.
Seizures induction	Stanojlović et al. 2009.
Induction of inflammation	da Cunha et al. 2010; da Cunha et al. 2012.
Overstimulation of NMDA receptors	Kamat et al. 2016; Kamat et al. 2013.
Disturbances in NO signaling	Hrncić et al. 2010; Hrncić et al. 2012*a*; Hrnčić et al. 2014*b*.

Figure 1. Major metabolic pathways of homocysteine and sulfur containing amino acids.

Figure 2. Personal research experience on the effects of homocysteine and homocysteine thiolactone compounds on cardiovascular system.

Figure 3. Mechanisms and pathways involved in homocysteine induced deleterious effects in cardiovascular system.

ERK - extracellular-signal-regulated protein kinase; IRAK - IL-1R-associated kinase; JNK - Jun kinases/SAPK; MAPK - mitogen-activated protein kinase; MMP - matrix metalloproteinases; MyD88 - myeloid differentiation primary response gene 88; NADPH oxidase - nicotinamide adenine dinucleotide phosphate-oxidase; NF-κB - nuclear factor kappa B; NMDA - N-methyl-D-aspartate; NOS - nitric oxide sinthase; NOS - nitric oxide; RBC - red blood cells; TIR - toll/interleukin-1 receptor; TIRAP - toll-Interleukin I receptor domain-containing adaptor protein; TLR4 - toll-like receptor 4; TRAM - TRIF-related adaptor molecule; TRIF - TIR domain-containing adaptor protein inducing interferon-b; TNF-α - tumor necrosis factor alpha


METHIONINE
ATP
Methionine adenosyltransferase
S-Adenosylmethionine (SAM)
CH3-acceptor - substrate
Methylated substrate
Methyltransferases
S-Adenosylhomocysteine (SAH)
H2O
SAH hydrolase
Adenosine
HOMOCYSTEINE
Serine
B6
Cystathionine β-synthase
CYSTATHIONE
B6
γ-cystathionase
α-Ketobutyrate
CYSTEINE
Sulfate
Urine
PROTEIN
TAURINE
GLUTATHIONE
Paraoxonase
ATP
AMP + 2Pi
Homocysteine thiolactone
Methionyl-tRNA synthetase
Choline
Betaine
Betaine homocysteine methyltransferase
Dimethylglycine
Methionine synthase
B12
Tetrahydrofolate
5,10-Methylen-tetrahydrofolate
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Methylenetetrahydrofolate reductase
Serine hydroxymethyltransferase
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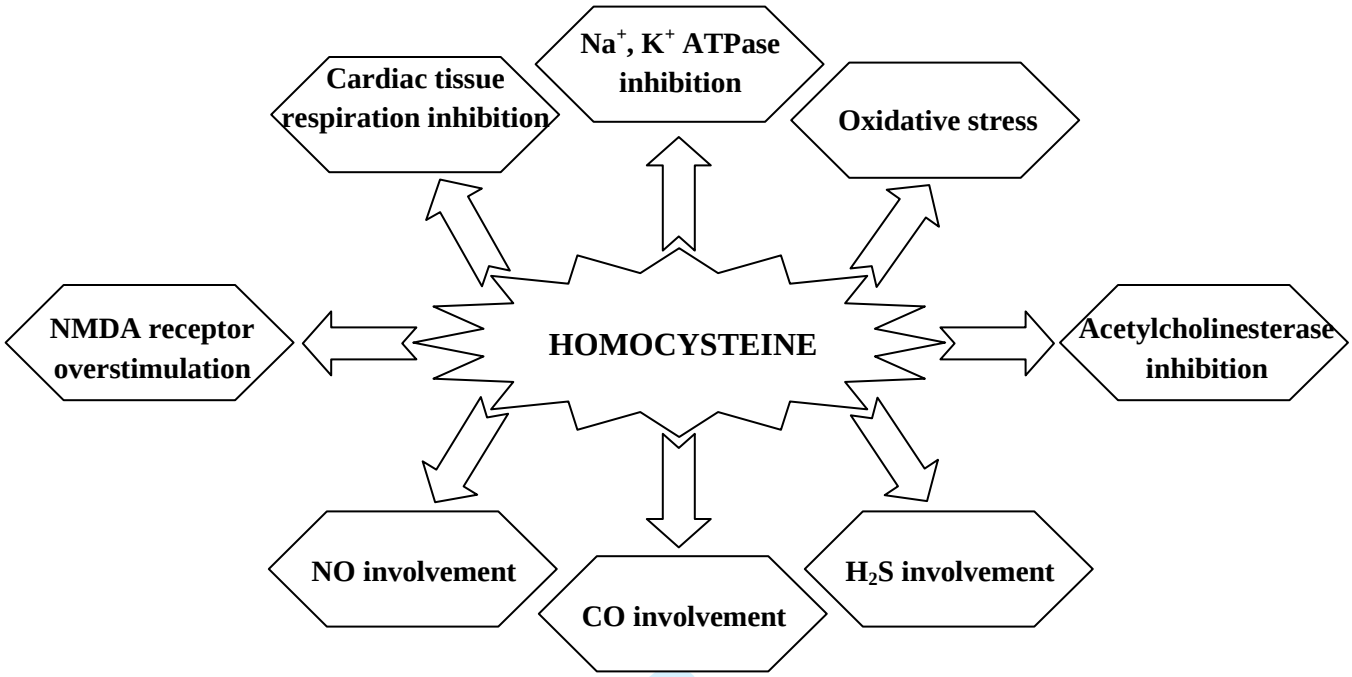
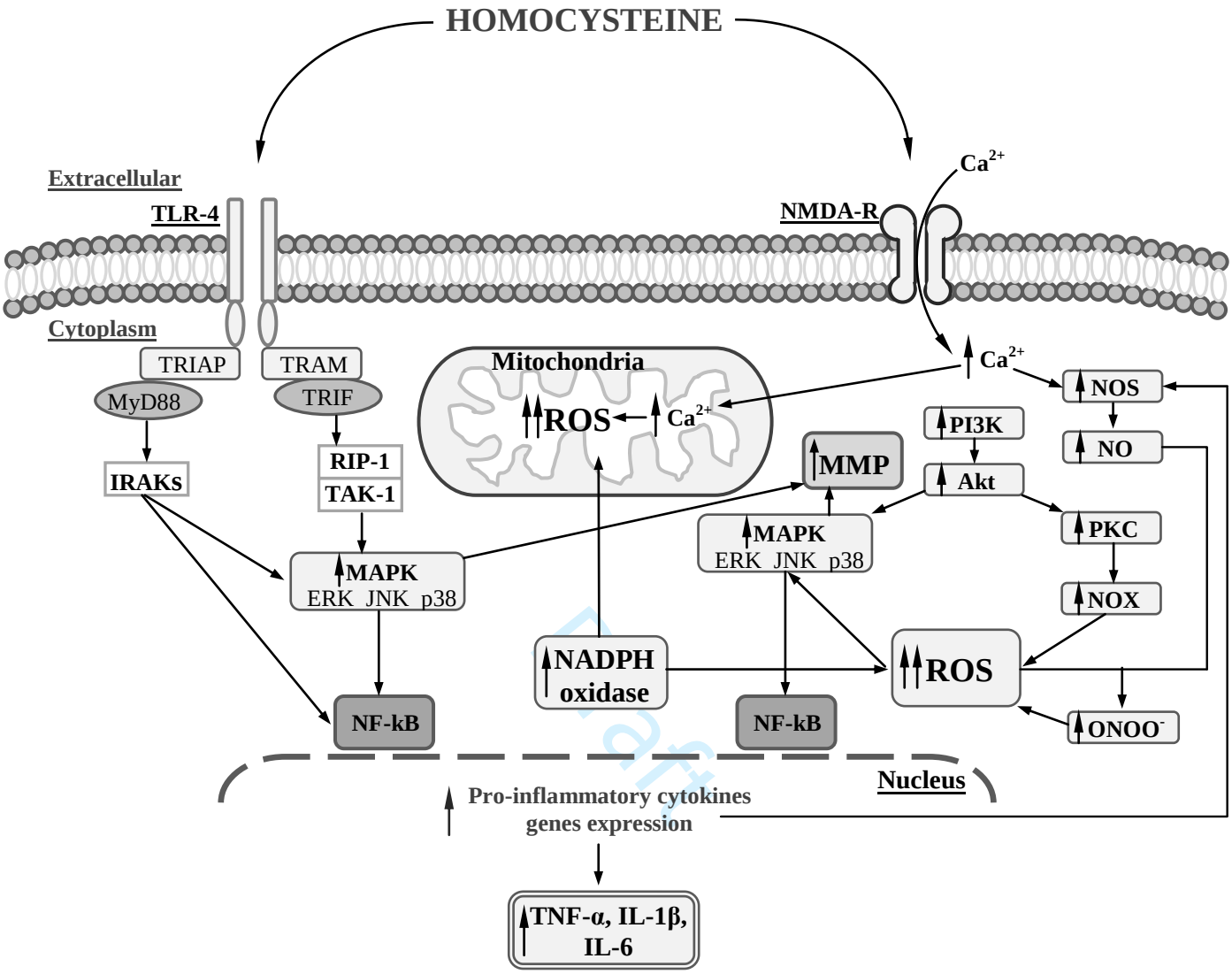
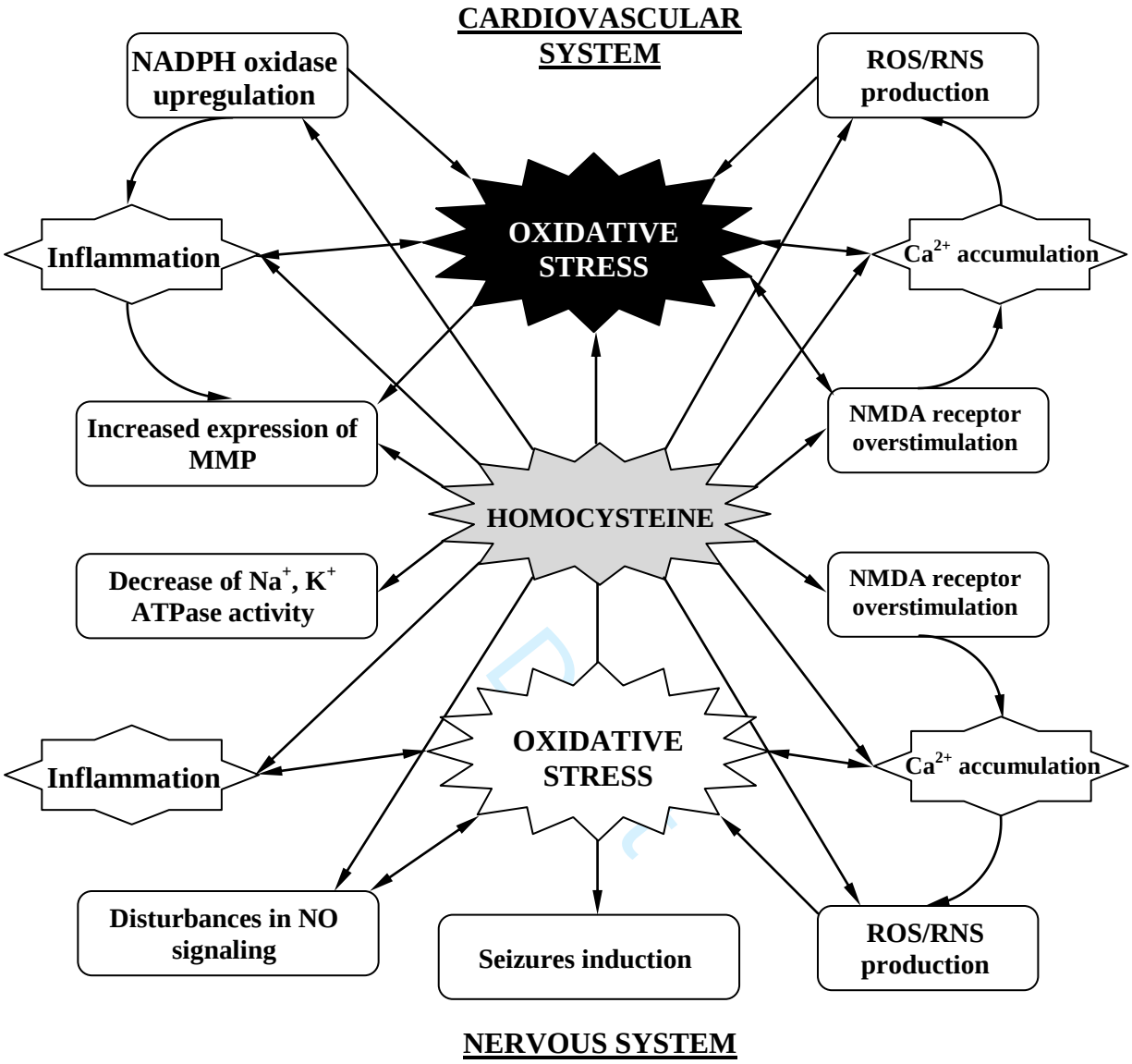
Serine
Glycine


Na+, K+ ATPase inhibition
Cardiac tissue respiration inhibition
Oxidative stress
NMDA receptor overstimulation
HOMOCYSTEINE
Acetylcholinesterase inhibition
NO involvement
CO involvement
H2S involvement


HOMOCYSTEINE
Extracellular
TLR-4
NMDA-R
Ca2+
Cytoplasm
TRIAP
TRAM
MyD88
TRIF
IRAKs
RIP-1
TAK-1
Mitochondria
ROS
Ca2+
Ca2+
NOS
NO
PI3K
Akt
MMP
PKC
MAPK
ERK JNK p38
MAPK
ERK JNK p38
NOX
NADPH
oxidase
ROS
NF-kB
NF-kB
ONOO-
Nucleus
Pro-inflammatory cytokines
genes expression
TNF-α, IL-1β,
IL-6


CARDIOVASCULAR SYSTEM
NADPH oxidase upregulation
ROS/RNS production
OXIDATIVE STRESS
Inflammation
Ca2+ accumulation
Increased expression of MMP
NMDA receptor overstimulation
HOMOCYSTEINE
Decrease of Na+, K+ ATPase activity
NMDA receptor overstimulation
OXIDATIVE STRESS
Inflammation
Ca2+ accumulation
Disturbances in NO signaling
Seizures induction
ROS/RNS production
NERVOUS SYSTEM