

Chapter 6

Homocysteine, Neurotoxicity and Hyperexcitability

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Abstract The research efforts worldwide have established the sulphur-containing amino acid homocysteine (Hcy) as a potent and independent risk factor (or risk marker) for a number of cardiovascular, as well as central nervous system disorders. This vasotoxic and neurotoxic agent interferes with fundamental biological processes and it is metabolized to homocysteine thiolactone, its highly reactive thioester. Hcy and its metabolites induced neuronal damage and cell loss through excitotoxicity and apoptosis. Our results showed that Hcy and Hcy thiolactone significantly affect neuronal cycles, EEG tracings and behavioral responses. After systemic administration, this naturally occurred substance led to the appearance of two different kinds of epileptic activity in adult rats. It has been suggested that Hcy thiolactone may be considered as an excitatory metabolite, capable of becoming a convulsant if accumulated to a greater extent in the brain. It was also found that changes in Na^+/K^+ -ATPase activity could be an important factor for the establishment of epileptic focus in Hcy-treated rats. Recently, we demonstrated functional involvement of NO signaling pathway in mechanisms of hyperexcitability caused by Hcy thiolactone. Acute ethanol treatment was shown in our study to decrease EEG power spectra and to represent one of the factors of the exogenous stabilization of brain excitability. Furthermore, our preliminary results showed that hypermethionine diet could contribute to these effects. Developed model of Hcy thiolactone-induced seizures in adult rats allows further investigations of mechanisms involved in Hcy's neurotoxicity and hyperexcitability.

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6.1 Introduction

Within the past several decades, the efforts of researchers have identified the amino acid homocysteine (Hcy) as a potent and independent, “new and emerging” risk factor for arteriosclerosis, as well as vasotoxic and neurotoxic agent involved in fundamental biological processes common to all cells. Therefore, it is also known as “cholesterol of the 21st century”.

Total plasma Hcy (tHcy) is defined as the pool of free Hcy, homocystine, Hcy-S-S-Cys disulfide, as well as protein-bound N- and S-linked Hcy, oxidized forms, and Hcy-thiolactone [1–3]. Under physiological conditions, less than 1 % of total Hcy is present in a free reduced form. About 10–20 % of total Hcy is present in different oxidized forms such as Hcy-Cys and homocystine, the Hcy dimer. Plasma tHcy levels are influenced by age, sex and genetic and lifestyle factors, as well as various pathologic conditions [1, 2, 4]. Hyperhomocysteinemia is present when tHcy concentration exceeds 10 μM .

Elevated tHcy is a recognized risk factor for cardiovascular disease [5–8] and has been linked to diseases of the aging brain including cognitive decline, vascular dementia and Alzheimer’s disease, cerebrovascular disease and stroke, including epilepsy. In addition, Hcy is pro-thrombotic and pro-inflammatory mediator [9, 10].

6.2 Homocysteine Metabolism and Its Implications

Metabolism of Hcy is regulated in order to achieve a balance between the remethylation and transsulfuration pathways which will maintain low levels of this potentially cytotoxic amino acid [11]. Hcy belongs to a group of molecules known as cellular thiols. Glutathione and cysteine, the most abundant cellular thiols, are considered to be “good” thiols, contrary to Hcy [12]. In the methylation pathway, Hcy acquires a methyl group to form methionine in a vitamin B12 dependent reaction catalyzed by the enzyme methionine synthase. The kidney, liver and eye lens have the capacity to convert Hcy to methionine through a vitamin B12-independent reaction catalyzed by betaine-Hcy S-methyltransferase (BHMT). On the contrary, the CNS lacks BHMT and therefore conversion of Hcy to methionine is completely dependent on the vitamin B12 and folate pathway.

Hcy condenses with serine to form cystathionine in an irreversible reaction catalyzed by the B6 containing enzyme – cystathionine beta-synthase, known as the transsulfuration pathway. Hcy catabolism requires vitamin B6 and as a consequence, alteration in folic acid and B vitamins status impairs Hcy biotransformation. These alterations result in the in the synthesis of cysteine, taurine and inorganic sulfates that are excreted in urine.

Elevation of Hcy levels is known to lead to metabolic conversion and inadvertent elevation of homocysteine thiolactone, a reactive thioester representing less than 1 % of total plasma Hcy. In all cell types, including endothelial and nerve cells, Hcy is metabolized to homocysteine thiolactone by methionyl-tRNA synthetase [13]. Homocysteine thiolactone causes lethality, growth retardation, blisters and somite

development abnormalities by oxidative stress, one important mechanism for toxicity to neural cells [14]. The highly reactive Hcy metabolite homocysteine thiolactone can be produced in two steps by enzymatic and/or non-enzymatic reactions in blood serum. Therefore, the ability to detoxify or eliminate homocysteine thiolactone is essential for biological integrity [13, 14].

6.3 Contribution of Homocysteine to Neurotoxicity

A number of studies provided evidences for a complex and multifaceted relation of homocysteinemia and CNS disorders. Hcy, as endogenous compound, is neurotoxic in supraphysiological concentrations. The hypothesis that relates Hcy to CNS dysfunction is based on its neuroactive properties. Adverse effects on brain functioning and debilities of high tissue Hcy concentrations appeared through oxidative stress and excitotoxicity-induced effects on neurons [15], and together with homocystinuria characterize patients with convulsions [16].

Hcy induces neuronal damage and cell loss through excitotoxicity and apoptosis, what could be a consequence of the inability of cerebral tissue to metabolize Hcy through the betaine and transsulfuration pathways, favoring Hcy accumulation in the nervous system [17]. High brain concentrations of either Hcy or its oxidised derivatives might alter neurotransmission [18]. An accumulation of Hcy (at synapses or in the extracellular space) would increase intracellular S adenosylhomocysteine (SAH), which is a potent inhibitor of many methylation reactions that are vital for neurological function including the O-methylation of biogenic amines. Methylation of myelin basic protein and reducing the synthesis of phosphatidyl choline, which can lead to disruption of the blood-brain barrier (BBB) are possible in absence of normal methylation patterns [19].

According to recent theory, Hcy toxicity is a consequence of covalent binding to proteins, interfering with protein biosynthesis, decreasing normal physiological activity of proteins thus modifying their functions in process called homocysteinyl-ation [13, 20]. Therefore, increased intracellular Hcy concentration is associated with both alteration of redox balance and post-translational protein modifications through *N*- and *S*-homocysteinyl-ation [21]. Moreover, some studies suggest that Hcy induces the expression of superoxide dismutase in endothelial cells, consumption of NO- and impaired endothelial vasorelaxation [22].

6.4 Hyperexcitability Induced by Homocysteine

6.4.1 *Experimental Models of Seizures*

Experimental models of epilepsy may be induced by manipulation of γ -aminobutyric acid (bicuculline, corasol, picrotoxin, benzylpenicillin sodium) [23] or by increasing cerebral excitatory neurotransmission. Experimental rat models of generalized

clonic-tonic seizure induced by metaphit [24, 25] and lindane [26–28] are suitable for the studies of epilepsy and preclinical evaluation of potential antiepileptic treatments. Almost four decades ago, Sprince et al. [29] described that high levels of Hcy, arising from excess dietary methionine, may induce epilepsy and lethality.

The fact that the elevated Hcy concentrations persists in damaged endothelial structures, during aging and antiepileptic-drug-therapy [30] justifies attention directed towards the examinations of homocysteine thiolactone effects. Namely, classical anticonvulsants (phenytoin, carbamazepine and valproic acid) lower plasma folate levels and increased significantly Hcy levels inducing epileptogenic brain and suboptimal control of seizures in the patients with epilepsy [31].

6.4.2 Two Types of Seizures in Adult Rats upon Homocysteine Thiolactone Administration

Stanojlović et al. [32] suggested that D, L-homocysteine thiolactone may be considered as an excitatory metabolite, capable of becoming a natural convulsant if accumulated to a great extent in the brain. Hyperhomocysteinemia in awake adult Wistar male rats induced recurrent unprovoked clonic-tonic convulsions and absence-like seizures, as well as specific electrical discharges. The seizure incidence, median seizure episode severity, median number of seizure episodes per rat, was significantly higher in all Hcy treated groups together with prolonged median latency to the first seizure [32]. Non-convulsive status epilepticus can occur from variety of causes including primarily generalized absence epilepsy, genetic origins (Wakayama or tremor epileptic rats) or pharmacologically (penicillin, pentylenetetrazole, γ -hydroxy-butyric acid) induced models [33]. The most puzzling phenomena in absence epilepsy are behavioral immobility during the active motor cortex and the occurrence of generalized spike-wave activity. SWDs may belong to the same class of phenomena such as sleep spindles. Sleep spindles are normally generated sleep rhythms that transform one, two or more spindle waves into the spike component of the SWD [34].

According to well known fact that rhythmic bursts of spikes represent an electrophysiological marker of a hyperexcitability, Folbergrova et al. [35] found very poor electroclinical correlation together with dissociation between electroencephalographic (EEG) pattern and motor phenomena in immature rats. The epileptogenic process is closely associated with the changes in neuronal synchronization. Non-lesion, non-convulsive, generalized epilepsy is characterized by brief episodes of unpredictable and unresponsive behavior with a sudden arrest accompanied by SWDs. This second type of spike-wave complexes had different shape. Bilateral, high-voltage synchronous, spindle-like electrical oscillations, phenomenon of paroxysmal electroencephalographic attacks, termed SWDs were associated with a sudden motor immobility and minor clinical signs like loss of responsiveness with rhythmic twitches of vibrissae or cervicofacial musculature were seen after i.p. administration of D, L-homocysteine thiolactone in adult rats.

Stanojlović et al. [32] found poor electro clinical correlations and dissociation of activity in rats. It is worth mentioning that electrographic seizure discharge was absent even during motor convulsions of grade 3 or 4, while on the contrary, EEG seizures without motor symptoms were regularly observed. Foremention EEG graphoelements were distinguishable from sleep spindles (10–16 Hz), regarding their frequency, duration, morphology (sleep spindles are more stereotyped than SWD waves) and moment of occurrence (SWD occurs during passive wakefulness vs. sleep spindle-like oscillation occurring during high amplitude delta activity).

6.4.3 Mechanisms of Homocysteine Convulsive Effects

There are several proposed mechanisms by which exposure to excess D,L-homocysteine thiolactone induces seizure [36].

Increased levels of Hcy and its metabolites could provoke seizures by increasing activation of some receptors like N-methyl-D-aspartate (NMDA) and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)/kainite ionotropic glutamate receptors [1]. These receptors are expressed in hippocampal pyramidal cells and may directly induce or drive these cells over the threshold for excitotoxic cell death. Overstimulation of these receptors triggers Ca^{2+} influx and intraneuronal calcium mobilization in the presence of glycine [37]. Increased cytosolic Ca^{2+} concentrations affect enzyme activities and synthesis of nitric oxide [38]. It should be noted that expression of NMDA receptor is not confined to neurons. Other cells, including endothelial cells from cerebral tissue, can express this receptor. Free radicals induce up-regulation of the NR1 subunit of the NMDA receptor, increasing the susceptibility of cerebral endothelial cells to excitatory amino acids, favoring BBB disruption [39]. Also, microglia is subject to toxic effects of Hcy [40]. Hcy could induce convulsions in adult, as well as in immature experimental animals throw modulating the activity of metabotropic glutamate receptors (mGluRs) [41].

Hcy was shown to enhance either the release or uptake of other endogenous excitatory amino acids [41]. It seems that Hcy exerts a direct excitatory effect comparable to the action of glutamate [16].

Rasic-Markovic et al. [42] investigated the effects of MK-801, NMDA antagonist, as well as, of ifenprodil, NR2B-selective NMDA antagonist in homocysteine thiolactone seizures and showed involvement of this mechanisms in homocysteine thiolactone induced epileptogenesis.

6.4.4 Involvement of nNOS Signaling Pathways in Homocysteine Hyperexcitability

Nitric oxide (NO) is a highly reactive messenger molecule synthesized in a number of tissues with key role in new form of interneuronal communication via modulating release of classical neurotransmitters and excitability status of neurons.

Hrnčić et al. [43] determined the role of NO in mechanisms of D, L homocysteine thiolactone induced seizures by testing the action of L-arginine (NO precursor) and L-NAME (NOS inhibitor) on behavioral and EEG manifestations of D, L homocysteine thiolactone induced seizures. Recently, the involvement of neuronal NO synthase (nNOS) in homocysteine thiolactone – induced seizures was determined using pharmacological inhibition of this enzyme by 7-nitroindazole, its selective inhibitor [44]. Congruent results with those obtained using non-selective inhibition were obtained.

6.4.5 Homocysteine and Na^+/K^+ ATPase Activity

The function of the Na^+/K^+ -ATPase is essential for generation of the membrane potential and maintenance of neuronal excitability [12]. Rašić-Marković et al. [45] demonstrated a moderate inhibition of rat hippocampal Na^+/K^+ -ATPase activity by D,L-homocysteine, which however expressed no effect on the activity of this enzyme in the cortex and brain stem. In contrast, D,L-homocysteine thiolactone strongly inhibited Na^+/K^+ -ATPase activity in cortex, hippocampus, and brain stem of rats structures affecting the membrane potential with deleterious effects for neurons. Hrnčić et al. [43] demonstrated that L-Arginine when applied alone, significantly increases the activity of Na^+/K^+ -ATPase activity in the hippocampus, the cortex and the brain stem and when applied prior to homocysteine thiolactone completely reversed the inhibitory effect of homocysteine thiolactone.

6.4.6 Modulation of Homocysteine-Induced Hyperexcitability

Complex relationship between sleep and epilepsy is still of special interest for neuroscientists since neurophysiological basis of that relation is far from being completely understood [46]. Sleep is a cyclic vital physiological process that makes one-third of human life [47]. It is estimated that about 20 % of the world's population still suffer from decrease in sleep time due to change of lifestyle and sleep disorders as the major causes. Recently, we have shown aggravation of seizure activity in homocysteine thiolactone – treated rats upon selective REM sleep deprivation [48].

Rašić-Marković et al. [49] examined the changes of total spectral power density in adult rats after ethanol alone and together with homocysteine thiolactone and it was found that action of ethanol on electrographic pattern was biphasic, with potentiation of epileptiform activity in one dose range and depression in another one. Low ethanol doses causing euphoria and behavioral arousal are associated with desynchronization of the EEG, decrease in the mean amplitude, and increase in the theta and alpha activity. Ethanol increased mean total spectral power density 15 and 30 min after administration, in all ethanol groups.

6.5 Conclusion

Results of aforementioned studies demonstrated that acute administration of homocysteine thiolactone significantly affected neuronal cycles, EEG tracing and behavioral responses. After systemic administration, this natural substance led to the disturbances in brain functioning and to the appearance of two different kinds of neuron network in adult Wistar rat males. It could be supposed that hyperhomocysteinemia might express similar effects on human brain activity. These effects are connected with stimulation of NMDA receptors, inhibition of the Na^+, K^+ -ATPase activity, and NO mediated signaling pathways during. Modulation of homocysteine – induced hyperexcitability was achieved by REM sleep deprivation and ethanol administration.

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