HOMOCYSTEINE: NEUROTOXICITY AND MECHANISMS OF INDUCED HYPEREXCITABILITY

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HOMOCISTEIN: NEUROTOKSIČNOST I MEHANIZMI INDUKOVANE HIPEREKSCITABILNOSTI

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ABSTRACT

Within the past four decades, investigators worldwide have established that the amino acid homocysteine (Hcy) is a potent, independent, novel and emerging risk factor for arteriosclerosis. In addition, Hcy is considered a vasotoxic and neurotoxic agent that interferes with fundamental biological processes common to all living cells. The aim of this article is to present data addressing central nervous system hyperexcitability in experimental models of homocysteine-induced seizures. These results demonstrate that acute administration of homocysteine and homocysteine-related compounds significantly affects neuronal activity, electroencephalographic (EEG) recordings and behavioural responses in adult Wistar male rats. It is suggested that hyperhomocysteinemia may generate similar effects on human brain activity. Homocysteine and D,L-homocysteine thiolactone are excitotoxic and elicit seizures through stimulation of NMDA receptors. In addition, they inhibit Na^+/K^+ -ATPase activity in the cortex, hippocampus and brain stem. Nitric oxide (NO) acts as an anticonvulsant in D,L-homocysteine thiolactone-induced seizures and prevents D,L-homocysteine thiolactone-induced inhibition of Na^+/K^+ -ATPase activity. EEG monitoring for the effects of ethanol on homocysteine-induced epilepsy support the idea that ethanol intake could represent one of the exogenous factors influencing brain excitability. Finally, it was found that homocysteine thiolactone significantly inhibits acetylcholinesterase (AChE) activity in rat brain tissue.

Keywords: homocysteine, CNS, hyperexcitability, rat

Abbreviations used:

AChE - acetylcholinesterase
BHMT - betaine-Hcy S-methyltransferase
CNS - central nervous system
EEG - electroencephalography
Hcy - homocysteine
NO - nitric oxide
SWDs - spike-wave discharges
tHcy - total plasma homocysteine

SAŽETAK

Četiri decenije istraživačkog rada širom sveta dovele su do ustanovljavanja semi-esencijalne sulfhidrilne aminokiseline homocisteina (Hcy) kao novog potentnog i nezavisnog faktora rizika za arteriosklerozu, kao i vazotoksične i neurotoksične supstance koja učestvuje u fundamentalnim biološkim procesima svojstvenim svim živim ćelijama. Cilj ovog rada je da prikaže dostupna saznanja na temu hiperekscitabilnosti centralnog nervnog sistema u eksperimentalnom modelu homocisteinom indukovane epilepsije. Rezultati pokazuju da akutna administracija homocisteina i homocisteinu-srodnih supstanci značajno utiče na nervnu aktivnost, elektroencefalografske (EEG) zapise i ponašanje odraslih mužjaka soja Wistar pacova, a smatra se da hiperhomocisteinemija može imati slične efekte i na aktivnost ljudskog mozga. Homocistein i D,L-homocistein tiolakton su ekscitotoksični i izazivaju epileptične napade stimulacijom NMDA receptora; oni indukuju značajnu inhibiciju aktivnosti Na^+/K^+ -ATPaze u korteksu, hipokampusu i moždanom stablu. Azot monoksid (NO) deluje kao antikonvulzant u D,L-homocistein tiolakton indukovanim epileptičnim napadima i vrši prevenciju D,L-homocistein tiolakton indukovane inhibicije aktivnosti Na^+/K^+ -ATPaze. EEG monitoring efekata etanola na homocisteinom indukovanu epilepsiju podržava ideju da akutni unos etanola može predstavljati jedan od faktora egzogenog uticaja na moždanu ekscitabilnost. Takođe je utvrđeno da D, L-homocistein tiolakton u značajnom procentu inhibira aktivnost acetilholinesteraze u mozgu pacova.

Ključne reči: homocistein, CNS, hiperekscitabilnost, pacov

Korišćene skraćenice:

AChE – acetilholin esteraza
BHMT - betain-homocistein S-metiltransferaza
CNS – centralni nervni sistem
EEG - elektroencefalografija
Hcy - homocistein
NO – azot monoksid
SWDs – "šiljak pražnjenja"
tHcy – ukupni plazma homocistein

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BACKGROUND

Within the past four decades, investigators worldwide have established that the amino acid homocysteine (Hcy) is a novel and independent risk factor for arteriosclerosis. In addition, Hcy is a vasotoxic and neurotoxic agent that interferes with the fundamental biological processes common to all living cells. Therefore, it has also been termed the “cholesterol of the 21st century.”

Metabolism of homocysteine is coordinately regulated to maintain a balance between remethylation and trans-sulfuration pathways, which are critical to maintaining low levels of this potentially cytotoxic sulphur containing amino acid (1). Homocysteine belongs to a group of molecules known as cellular thiols and is considered a “bad” thiol. Glutathione and cysteine, the most abundant cellular thiols, are considered to be “good” thiols (2). In the methylation pathway that occurs in all tissues, homocysteine acquires a methyl group to form methionine in a vitamin B12 dependent reaction, which is catalysed by methionine synthase. The second substrate required by methionine synthase is 5-methyltetrahydrofolate, which is formed by the reduction of 5,10-methylenetetrahydrofolate. This reaction is catalysed by the enzyme methylenetetrahydrofolate reductase (MTHFR). The TT genotype of this enzyme causes thermolability and reduces enzyme activity, which impairs the formation of 5-methyltetrahydrofolate. This reduced enzyme activity explains why this genotype is associated with increased Hcy levels when the folate status is relatively low. However, liver, kidney and the lens of the eye have the ability to convert Hcy to L-methionine through a vitamin B12-independent reaction catalysed by betaine-Hcy S-methyltransferase (BHMT). The CNS lacks BHMT and it is completely dependent on the folate and vitamin B12 pathway for the conversion of Hcy to L-methionine.

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

Elevation of homocysteine levels is known to lead to the metabolic conversion and inadvertent elevation of homocysteine thiolactone, which is a reactive thioester representing less than 1% of total plasma homocysteine. In all cell types, from bacteria to human, homocysteine is metabolised to homocysteine thiolactone by methionyl-tRNA synthetase (3). Homocysteine thiolactone causes lethality, growth retardation, blisters and abnormalities in somite development by oxidative stress, which is one of important mechanisms for its toxicity to neuronal cells (4). The highly reactive and toxic homocysteine metabolite, homocysteine thiolactone, can be produced in two steps by enzymatic and/or non-enzymatic reactions in blood serum. The ability to detoxify or eliminate homocysteine thiolactone is essential for biological integrity (3, 4).

Total plasma Hcy (tHcy) consists of a pool of free homocysteine, homocystine, Hcy-S-S-Cys disulphide, protein bound N- and S-linked Hcy as well as their oxidised forms and Hcy-thiolactone (5-7). Under physiological conditions, less than 1% of the total Hcy is present in a free reduced form. Approximately 10–20% of total Hcy is present in different oxidised forms, such as Hcy-Cys and homocystine, which is a Hcy dimer. Plasma tHcy levels are affected by genetic, physiologic (age and sex) and lifestyle factors as well as various pathologic conditions (5, 6, 8). Hyperhomocysteinemia is defined as an elevated plasma total homocysteine (tHcy) concentration (>15 μ M).

Elevated tHcy is a recognised risk factor for cardiovascular disease (9) and has been linked to neurological diseases during aging, such as cognitive declines, cerebrovascular disease and stroke, vascular dementia and Alzheimer’s disease. In addition, elevated tHcy levels are linked with pathological brain functioning, such as mental retardation, depression, schizophrenia and memory impairment. Hcy is also a pro-thrombotic and pro-inflammatory factor, vasodilatation impairing substance and an endoplasmatic reticulum stress inducer (10, 11).

Mitrovic et al. (12) evaluated total plasma homocysteine in patients with angiographically confirmed coronary artery disease. In addition, they investigated the effects of homocysteine lowering therapy on endothelial function, carotid wall thickness and myocardial perfusion. Their study found that homocysteine levels decreased significantly (34%) with folic acid therapy and that endothelium function improved by 27% with this treatment. However, carotid structure and myocardial perfusion did not show any significant improvement in patients with confirmed coronary artery disease (13). Djuric et al. (14, 15) also investigated the link between homocysteine and folic acid, demonstrated folic acid induced coronary vasodilation and decreased oxidative stress in the isolated rat heart.

HOMOCYSTEINE AND NEUROTOXICITY

Despite several theories, a complete understanding of Hcy toxicity remains unclear. In particular, the hypothesis that relates homocysteine to CNS dysfunction by way of overt neurotoxicity is based on the neuroactive properties of homocysteine. Elucidating the link between homocysteine and CNS dysfunction is vital for improving the treatment of homocysteine related CNS disorders. Hcy adversely affects brain functioning (i.e., mental retardation, dementia and memory impairment) and high tissue concentrations cause oxidative stress and excitotoxicity in neurons (16). When these symptoms are combined with homocystinuria, patients often present with convulsions (17). These studies provide evidence for a complex and multifaced relationship between homocysteinemia and CNS disorders. Hcy is an endogenous compound that is neurotoxic at supraphysiological concentrations and induces neuronal damage and cell loss through excitotoxicity and apoptosis. These CNS

pathologies could be a direct consequence of the inability of cerebral tissue to metabolise Hcy through the betaine and transsulfuration pathways, thereby favouring Hcy accumulation in the nervous system (18). High brain concentrations of either Hcy or its oxidised derivatives have been shown to alter neurotransmission (19). The accumulation of homocysteine (at synapses or in the extracellular space) increases 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 blood-brain barrier (BBB) disruption, are a few pathological processes that are possible in the absence of normal methylation patterns (20).

It has been recently suggested that Hcy toxicity is a consequence of its covalent binding to proteins, which interferes with protein biosynthesis and decreases the normal physiological activity of proteins. This modification of protein function is a process called homocysteinylation. Transthyretin is a plasma protein that is modified through homocysteinylation (3, 21). Other reports suggest that Hcy exerts its toxicity through the induction of endoplasmic reticulum (ER) stress. Increased intracellular Hcy concentrations are associated with both the alteration of redox balance and post-translational protein modifications through N- and S-homocysteinylation (22). Other studies suggest that Hcy induces the expression of superoxide dismutase in endothelial cells, which leads to the consumption of NO and impairs endothelial vasorelaxation (23).

HOMOCYSTEINE AND SEIZURES

Experimental models of generalised clonic-tonic seizures induced by metaphit (1-[1(3-isothiocyantophenyl)-cyclohexyl] piperidine) (24, 25) and lindane (γ -1,2,3,4,5,6-hexachlorocyclohexane) (26, 27) are widely used for the study of epilepsy and the preclinical evaluation of potential antiepileptic treatments. In contrast to audiogenic epilepsy induced by metaphit with its typical and characteristic three degrees of intensity, the lindane model is similar to Hcy-induced epilepsy with a wide spectra of behavioural manifestations (e.g., hot plate reaction and Kangaroo position) incorporated into the rating scale from grades 0 - 4. Almost four decades ago, Sprince et al. (28) described that elevated levels of homocysteine arising from excess dietary methionine may induce epilepsy and lethality. The fact that increased homocysteine concentrations damage endothelial structures and the blood coagulation system during aging and in patients on antiepileptic drugs (29) justifies the attention directed toward the examination of homocysteine's neural effects.

Furthermore, experimental models of epilepsy may be induced by manipulations of γ -aminobutyric acid (e.g., bicuculline, corasol, picrotoxin and benzylpenicillin sodium) (30). In contrast, epilepsy can be induced by an opposite

mechanism such as by increasing cerebral excitatory neurotransmission. There are several proposed mechanisms by which exposure to excess D,L-homocysteine or D,L-homocysteine thiolactone induce seizures. The most reactive natural amino acid is homocysteic monocarboxylic, which is sulphur-containing and its homocysteic sulphinic acid derivatives are excitatory amino acids that have been proposed as candidates for brain excitatory neurotransmitters (31). However, answering the question as to whether D,L-homocysteine thiolactone should be included in the list of epileptogenic factors according to behavioural and electrophysiological responses will require additional studies and future human therapeutic trials. Increased levels of Hcy and its oxidised forms can provoke seizures by increasing activation of certain neuronal receptors such as N-methyl-D-aspartate (NMDA), α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)/kainate ionotropic glutamate receptors or group I and group III metabotropic glutamate receptors (5). All of these glutamate 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 cytoplasmic calcium pulses, Ca^{2+} influx and intraneuronal calcium mobilisation in the presence of glycine (32). Increased cytosolic Ca^{2+} concentrations affect enzyme activities and the synthesis of nitric oxide, which is a retrograde messenger that overstimulates excitatory amino acid receptors including glutamate, thereby lowering the convulsive threshold (33). However, expression of the NMDA receptor is not confined to neurons. Other cell types, including endothelial cells from cerebral tissue, can express this receptor. Free radicals induce the up-regulation of the NR1 subunit of the NMDA receptor, thereby increasing the susceptibility of cerebral endothelial cells to excitatory amino acids, which can lead to blood brain barrier disruption (34). Microglia are also subject to the toxic effects of Hcy (35). Hcy can induce convulsions in adults and in immature experimental animals through modulating the activity of metabotropic glutamate receptors (mGluRs) (36). Kubova et al. (37) stated that the main epileptic phenomenon in homocysteine thiolactone treated rats was emprosthotonic, flexion seizures, which are observed in young animals but never seen in rats older than 25 days. Homocysteine was shown to elicit minor (predominantly clonic) and major (generalised tonic-clonic) seizures during ontogenesis in immature Wistar rats (36, 37). It seems that Hcy exerts a direct excitatory effect comparable to the action of glutamate (17). In addition, Hcy has been shown to enhance either the release or uptake of other endogenous excitatory amino acids (36).

Classical anticonvulsants such as phenytoin, carbamazepine and valproic acid lower plasma folate levels and significantly increase homocysteine levels, which induces epileptogenesis and reduces the control of seizures in patients with epilepsy (38). On the other hand, increasing excitability with this native amino acid further contributes to epileptogenesis and disturbs the balance between excitation and inhibition in

the brain. Following the work of Djuric's study, Stanojlovic et al. administered homocysteine (i.p.) to adult rats, and for the first time, convulsive and non-convulsive seizures were recorded (39). This form of epilepsy was followed by two-wave patterns of seizures with one replacing the other. It has been suggested that D,L-homocysteine thiolactone may be considered as an excitatory metabolite, which is capable of becoming a natural convulsant if accumulated to a great extent in the brain (39). Hyperhomocysteinemia in awake adult male Wistar rats induced a generalised seizure disorder characterised by recurrent unprovoked clonic-tonic convulsions and absence-like seizures as well as epileptic electrical discharges. In addition to a prolonged median latency to the first seizure, the seizure incidence, median seizure episode severity and median number of seizure episodes per rat were significantly higher in all Hcy treated groups (39). Non-convulsive status epilepticus can occur from a variety of causes, including primarily generalised absence epilepsy, genetic origins (Wakayama or tremor epileptic rats) or pharmacologically (penicillin, pentylentetrazole or γ -hydroxy-butyric acid) induced models (40). Behavioural immobility during motor cortex activation and the occurrence of generalised spike-wave activity are among the most puzzling phenomena in absence epilepsy. According to one of numerous hypotheses addressing the development of spike-wave discharges (SWDs), these electrographic discharges may belong to the same class of phenomena such as sleep spindles. Sleep spindles are normally generated sleep rhythms that transformation one, two or more spindle waves into the spike component of the SWD (41). Cortico-thalamo-cortical oscillatory network connections may play a pacemaker role in the electrogenesis of oscillations and primarily represent the neurophysiological basis underlying the initiation and propagation of SWD (40).

In keeping with the well-known fact that rhythmic bursts of spikes represent an electrophysiological marker of a hyperexcitability, Folbergrova et al. (42) found a very poor electroclinical correlation between electroencephalographic (EEG) patterns and motor phenomena in immature rats. The epileptogenic process is closely associated with the changes in neuronal synchronisation, and non-convulsive, generalised epilepsy is characterised by brief episodes of unpredictable and unresponsive behaviours with a sudden arrest accompanied by SWDs. However, the spike-wave complexes of SWDs have a unique shape. Bilateral, high-voltage, synchronous, spindle-like electrical oscillations, which are common phenomenon during paroxysmal EEG attacks, are termed SWDs. These are associated with sudden motor immobility and minor clinical signs, such as the loss of responsiveness with rhythmic twitches of vibrissae or cervicofacial musculature and are seen after i.p. administration of D,L-homocysteine thiolactone in adult rats. Interestingly, absence-like animals showed no reaction to these different stimuli (e.g., audiogenic, tactile).

Stanojlović et al. (39) found poor electro-clinical correlations in Hcy treated rats. It is worth mentioning that electrographic seizure discharges were absent even during motor convulsions of grade 3 or 4. On the contrary, EEG seizures

without motor symptoms were regularly observed. These aforementioned EEG signatures were distinguishable from sleep spindles (10-16 Hz) in regard to their frequency, duration, morphology (sleep spindles are more stereotyped than SWD waves) and moment of occurrence. For example, SWDs occur during passive wakefulness, while sleep spindle-like oscillations occur during high amplitude delta activity (43). The poor prognosis for the Hcy group of animals appears to be a result of the combined systemic damage caused by continuous seizure activity as well as the excitotoxic and neurotoxic effects of homocysteine accompanied by the action of free radicals, including oxidative brain damage (42, 44). In most of the related studies, it is not quite clear whether the observed effects are due to homocysteine itself or to homocysteine metabolites. Notably, all of the above mentioned mechanisms induce cell death (45) and result in a high mortality rate (100%).

The basic function of the Na^+/K^+ -ATPase is to maintain the high Na^+ and K^+ gradients across the plasma membrane of animal cells and it is essential for the generation of the membrane potential and maintenance of neuronal excitability (2). In addition, it has been demonstrated that there is a moderate inhibition of rat hippocampal Na^+/K^+ -ATPase activity by D,L-homocysteine, which is not observed in the cortex and brain stem (46). In contrast, D,L-homocysteine thiolactone strongly inhibits Na^+/K^+ -ATPase activity in cortex, hippocampus and the brain stem of rats, thereby affecting the membrane potential with deleterious effects for neurons. Considering that hyperhomocysteinemia increases formation of superoxide and hydrogen peroxide and that excitotoxicity indirectly provokes increased intracellular free radicals production, it is feasible that oxidative stress may also be associated with the CNS injury caused by homocysteine and D,L-homocysteine thiolactone (47).

Otherwise, nitric oxide (NO) is a highly reactive second messenger molecule synthesised in a number of tissues. It serves a key role in interneuronal communications via modulating release of classical neurotransmitters and influencing the excitability status of neurons (33). It is produced from L-arginine by the action of a family of enzymes known as NO synthases (NOS). Neural (nNOS) and endothelial NOS (eNOS) are Ca^{2+} /calmodulin-dependent enzymes, while inducible NOS (iNOS) shows Ca^{2+} -independent properties. N-nitro-L-arginine methyl ester (L-NAME) is a non-selective NOS inhibitor commonly used to decrease NO levels. Recently, it has been determined that NO serves a role in the mechanisms underlying D,L homocysteine thiolactone induced seizures. This was demonstrated by testing the action of L-arginine (NO precursor) and L-NAME (NOS inhibitor) on behavioural and EEG manifestations of D,L homocysteine thiolactone induced seizures (48). Following Djuric's study, Hrnčić et al. (48) showed functional involvement of NO in the convulsive activity of D,L-homocysteine thiolactone induced seizures in adult rats. Pretreatment with L-NAME in a dose-dependent manner increased seizure incidence and severity and shortened latency time to the first seizure following injection with a subthreshold dose of D,L-homo-

cysteine thiolactone (5.5 mmol/kg, i.p.). Nitro indazole is an inhibitor of neuronal nitric oxide synthase that attenuates pilocarpine-induced seizures (49). NO has been shown to modulate NMDA receptor activity by interacting with the –SH group of its redox modulatory site via S-nitrosylation. This modification results in the downregulation of this receptor complex (19) and prevents the neurotoxic effects of an excessive Ca^{2+} influx during homocysteine induced overstimulation of NMDA and mGluRs I receptors. In addition, Kim et al. (50) demonstrated that NO ameliorates homocysteine's adverse effects of S-nitrosylation in cultured rat cortical neurons. Moreover, NO induces a reduction of glutamate by activation of glial cells. Many experimental studies have demonstrated co-localisation of NOS and GABA and have suggested that basal NO levels induce a depression of inhibition, while high concentrations of NO increase GABA release. In contrast to homocysteine, which increases oxidative stress by the production of reactive oxygen species, NO can act as a neural protector. This neuroprotective property is due to the formation of S-nitroso-L-glutathione, which is an antioxidant and NO scavenging molecule (51). However, the role of NO in epileptogenesis has been studied in different experimental models and reported results are highly contradictory. Namely, the proconvulsive role of NO has been demonstrated in the lindane model of convulsions and several others (52). Thus, it seems that the activity of NO depends on the animal strain, the seizure model employed and the type and dose of drugs used in order to modify cerebral NO levels.

Furthermore, it has been demonstrated that when L-arginine is applied alone, it significantly increases the activity of Na^+/K^+ -ATPase activity in the hippocampus, the cortex and the brain stem. However, when L-arginine is applied prior to D,L homocysteine thiolactone (8 mmol/kg), this completely reverses its inhibitory effect (48). The same holds true for its effects on Mg^{2+} -ATPase activity in the rat cortex and the brain stem. L-NAME increases Na^+/K^+ -ATPase activity in the cortex and the brain stem but not in the hippocampus. When L-NAME was administered prior to homocysteine thiolactone (5.5 mmol/kg), it increased the activity of both Na^+/K^+ -ATPase and Mg^{2+} -ATPase in the rat cortex and the hippocampus.

Changes in total spectral power density after ethanol alone and together with D,L-homocysteine thiolactone in adult rats were examined (53). Recording electrical activity from the brain represents a measure of both brain function and dysfunction. Ethanol is used as a social substance and is the second most widely used psychoactive substance in the world after caffeine. The influence of ethanol on the central nervous system depends on the dose, drinking pattern, tolerance and other factors. While chronic ethanol consumption is followed by a series of seizures during the withdrawal period, acute ethanol intake exerts mainly inhibitory effects on the CNS and is usually associated with an increase of seizure threshold (54). Rasic-Markovic et al. (53) found that ethanol's action on electrographic pattern is biphasic, which is char-

acterised by potentiation of epileptiform activity in one dose range and depression in another one. Low ethanol doses causing euphoria and behavioural arousal are associated with desynchronisation of the EEG, decreases in the mean amplitude and increases in theta and alpha activity. In addition, ethanol increases mean total spectral power density 15 min and 30 min after administration. Ethanol affects voltage-gated ion channels, second messenger systems and a variety of different neurotransmitter systems such as glycine, acetylcholine as well as monoamines and neuropeptides systems (55). Two major amino acid neurotransmitter systems, GABA and glutamate, as well as aspartate are affected by ethanol. Acute administration of ethanol inhibits NMDA-induced Ca^{2+} influx, cyclic GMP production, neurotransmitter release and reduces NMDA-evoked neurotoxicity (56, 57).

Pre-treatment with MK-801, which is a NMDA receptor antagonist, showed a tendency to reduced the incidence of convulsions, latency to the first seizure onset and the severity of seizure episodes; however, there was no statistical significance when compared to the D,L-homocysteine thiolactone treated group. Nevertheless, the median number of seizure episodes was significantly decreased by MK-801 when compared to the D,L-homocysteine thiolactone treated group. On the other hand, ifenprodil, which is another type of NMDA receptor antagonist, decreased the latency to the first seizure onset and increased the median number of seizure episodes. The majority of seizure episodes in the ifenprodil (72.1%) and MK-801 (73.1%) groups were significantly different compared to the D,L-homocysteine thiolactone treated group (36.0%). Our findings suggest that D,L-homocysteine thiolactone induces seizures through the stimulation of NMDA receptors in the central nervous system but other mechanisms (i.e., NO signalling) may also be involved (58).

Finally, limited data exist in the literature regarding the effects of homocysteine and D,L-homocysteine thiolactone on the activity of the acetylcholinesterase (AChE) enzyme in the blood, but practically no data exist regarding the influence of these compounds on this enzyme in the brain and heart. Recent results showed a significant reduction in AChE activity in all tissues obtained from rats treated with D,L-homocysteine thiolactone compared to the enzyme activity of the control group. In addition, these results also showed that the blood enzyme activity was the lowest (12%) after treatment, while the enzyme activity was slightly higher in the brain (27.8%) and heart samples (86.3%). Therefore, it was concluded that D,L-homocysteine thiolactone significantly inhibited AChE activity in the heart and brain tissue but not in the blood of the rat (59).

Overall, these studies clearly demonstrate that acute administration of homocysteine and especially D,L-homocysteine thiolactone elicit seizures in adult rats, affect neuronal activity, EEG recordings and behavioural responses. These effects have been linked to the stimulation of NMDA receptors, inhibition of the Na^+/K^+ -ATPase, inhibition of AChE activity and the functional involvement of NO during D,L-homocysteine thiolactone induced seizures in adult rats.

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While dysfunction of the prefrontal cortex has been repeatedly implicated in the pathophysiology of schizophrenia, the role of serotonin in this brain region in schizophrenia is unclear. We therefore examined the effects of local serotonin depletion in the medial prefrontal cortex on psychotomimetic drug-induced locomotor hyperactivity and prepulse inhibition, two animal models of aspects of schizophrenia. Pentobarbital-anaesthetised (60 mg/kg, i.p.) male Sprague-Dawley rats were stereotactically micro-injected with 0.5 μ l of a 5 μ g/ μ l solution of the serotonin neurotoxin 5,7-dihydroxytryptamine into the medial prefrontal cortex. Two weeks after the surgery, rats underwent behavioural testing. When compared to sham-operated controls, rats with medial prefrontal cortical lesions did not show changes in either psychotomimetic drug-induced locomotor hyperactivity or prepulse inhibition. However, following the administration of the serotonin neurotoxin into the medial prefrontal cortex, the concentration of serotonin was reduced by 60%. These results suggest that serotonin depletion in the medial prefrontal cortex does not lead to dysregulation of psychotomimetic drug-induced locomotor hyperactivity and does not