Available online at www.sciencedirect.com
SCIENCE DIRECT®
Neuroscience Letters 373 (2005) 119–124
Neuroscience Letters
www.elsevier.com/locate/neulet

Enhanced hippocampal long-term potentiation in rats after chronic exposure to homocysteine

Louisa A. Christie[a], Gernot Riedel[a], Sami A. Algaidi[a], Lawrence J. Whalley[b], Bettina Platt[a,*]

[a] *School of Medical Sciences, College of Life Sciences and Medicine, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, Scotland, UK*
[b] *Clinical Research Centre, Department of Mental Health, Royal Cornhill Hospital, University of Aberdeen, Aberdeen AB25 2ZH, UK*

Received 18 August 2004; received in revised form 23 September 2004; accepted 30 September 2004

Abstract

Homocysteine (HCY) is a sulphur-containing amino acid, which has been linked to neurodegenerative diseases such as Alzheimer's disease, and is widely reported to enhance vulnerability of neurons to oxidative, excitotoxic and apoptotic injury via perturbed calcium homeostasis, activation of *N*-methyl-D-aspartate (NMDA) and metabotropic glutamate (mGlu) receptors. The present study was undertaken to investigate the effects of HCY on long-term potentiation (LTP) and synaptic transmission after chronic 4-week systemic exposure to HCY in adult rats, and possible longer-term effects of HCY 4 weeks after exposure had ended. Contrary to expectation, LTP was enhanced, not retarded after chronic HCY exposure relative to controls. Basic synaptic transmission was not affected at this time point. However, after the 4-week wash out period, a decrease in speed of basic synaptic transmission emerged, and LTP was still partially enhanced, particularly for time points >30 min post-tetanus.

In summary, we provide first evidence for sustained HCY-induced changes in hippocampal plasticity and a slow-onset disruption in synaptic transmission. These changes may reflect the suggested (excito-)toxicity of HCY and its putative contribution to neurodegenerative disease.

Keywords: LTP; Synaptic plasticity; Hippocampus; Nutrition; Neurodegeneration; Alzheimer's disease

Alzheimer's disease (AD) is the most common cause of cognitive decline in the elderly, and affects around 12 million patients worldwide. Brain regions relevant for learning and memory such as the hippocampus and adjacent areas, are particularly affected in AD. In addition to genetic pre-dispositions accounting for less than 10% of AD cases, exogenous factors such as toxins, brain trauma and nutrition have been discussed to contribute to both onset and progression of AD. In particular, there is increasing evidence for the involvement of dietary factors such as Vitamin B deficiency and increased homocysteine (HCY) levels in adult neurological disorders [23,25]. HCY is a non-essential sulphur-containing amino acid with putatively toxic properties [13]. Significantly higher total HCY levels in plasma along with a reduction of folate and B12 levels in serum and spinal fluids were reported in patients with AD, in comparison with neurologically normal age matched controls [3]. The two main pathways that catabolise HCY, remethylation and transsulfuration, are both vitamin-dependent, and interestingly complete transsulfuration only occurs in non-neuronal tissues and nervous tissue, is therefore, thought to lack this method of removing HCY [4].

In a recent animal study, a model of chronic hyperhomocysteinemia in rats provoked a memory deficit in the Morris water maze task [20]. Results showed that rats subcutaneously injected twice a day, over 3 weeks, with weekly increases in HCY doses (0.3/0.4/0.6 mmol/kg), had impaired memory of the platform location and in the working memory task, needed more time to find the platform in comparison with controls. In addition, it was shown that pre-treatment with Vitamins E and C actually prevents impairment of memory in a model of hyperhomocysteinemia [15].

It is, however, still unclear whether elevated HCY is simply a consequence of neurodegenerative disease, or

* Corresponding author. Tel.: +44 1224 555741; fax: +44 1224 555719.
E-mail address: b.platt@abdn.ac.uk (B. Platt).

0304-3940/$ – see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2004.09.072

whether it is indeed a causal factor. HCY has been suggested to act as a partial agonist at the glutamate binding site of the NMDA receptor in the presence of high glycine concentration [11] and therefore, the nervous system may be particularly sensitive to HCY as it may promote excitotoxicity via stimulation of NMDA receptors and damage neuronal DNA triggering apoptosis [13]. Excess intracellular Ca^{2+} and the resulting disturbances in Ca^{2+} homeostasis are closely linked to oxidative stress, and result in the generation of nitric oxide (NO) and accumulation of reactive oxygen species (ROS). Increased mitochondrial ROS production was indeed found to occur in cultured neurons exposed to HCY [9]. In vitro studies demonstrate HCY-mediated cell death can be prevented by co-administration with superoxide dismutase (SOD) and catalase or with catalase alone suggesting that formation of hydrogen peroxide contributes to HCY-mediated cell death [7].

Thus, since excitotoxicity followed by oxidative stress and ROS are major contributors in many neurodegenerative diseases such as AD [17,23], these pathways may explain how HCY is involved in, or even causes neurodegeneration [22]. In the present study, we set out to investigate whether the reported link between neurodegeneration, memory deficits and enhanced HCY levels manifests itself in alterations of basic synaptic transmission and neuronal plasticity in the rat hippocampus. As in previous studies, we used the well-established model of long-term potentiation (LTP) to determine changes in hippocampal plasticity [14]. Putative changes were assessed during HCY exposure and after a period of recovery. For this, male hooded Lister rats (360–370 g, Fig. 1) were anaesthetised with intraperitoneal injection of Avertin (tribromoethanol) and underwent surgery involving insertion of osmotic minipumps (Model 2ML4, Alzet, Palo Alto, CA) subcutaneously in the abdominal wall. These contained 2 ml of either vehicle (sterile filtered ddH_2O; controls, $n = 4$) or 100 mM HCY, released over 4 weeks

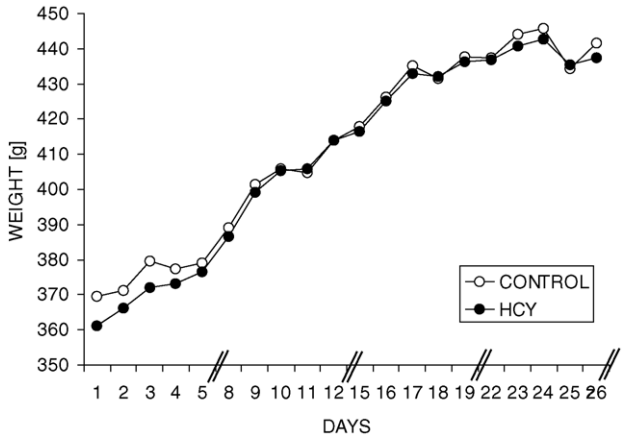

Fig. 1. Weight chart illustrating initial average weight and weight gain of control ($n = 4$) and HCY exposed animals ($n = 8$) during the 4-week minipump infusion period. The weight gain of the HCY animals did not differ between these groups. The symbol "//" indicates weekend breaks when animals were not weighed.

(equivalent to ∼1 mg HCY/day). D,L-Homocysteine was obtained from Sigma–Aldrich (Dorset, UK), dissolved in water and sterile filtered prior to use. This dose was chosen based on initial tests indicating that higher HCY concentrations did not stay in solution for longer than a few hours.

Weight gain of all groups was closely monitored and recorded over the period of exposure. Rats were taken for electrophysiological analysis during week 4 (HCY group 1, $n = 4$) of minipump infusion, or during week 8 (HCY group 2, $n = 4$), i.e. 4 weeks after minipump contents were exhausted. All animal procedures followed institutional guidelines and were approved by the Home Office. For slice preparation, animals were terminally anaesthetised with halothane, decapitated, the brain quickly removed and placed in ice-cold artificial cerebrospinal fluid (aCSF) containing (in mM): 129.5 NaCl, 1.5 KCl, 1.3 $MgSO_4$, 2.5 $CaCl_2$, 1.5 KH_2PO_4, 25 $NaHCO_3$ and 10 glucose (pH 7.4, continuously gassed with 95% O_2/5% CO_2). Hippocampal slices (400 μm) were prepared using a McIllwain tissue chopper and allowed to equilibrate in pre-warmed (32 °C) aCSF for at least 1 h following the preparation.

Field population spikes were elicited via a monopolar stimulation electrode (WPI, 0.5 MΩ) placed in the Schaffer collateral fibres, and recorded via an aCSF-filled borosilicate glass electrode (3–7 MΩ) positioned in the CA1 pyramidal cell body layer. Input/output (I/O) curves of basic synaptic transmission were determined initially, the stimulation intensity being increased in a stepwise fashion (stimulus duration: 100 μs), until the signal reached saturation. Subsequent LTP experiments were performed at 50–60% of this maximum, and stable baseline responses (±10%) were recorded every 30 s for at least 10 min. Slices with unstable signals were excluded. To induce LTP, a theta (5 Hz) tetanus (150 bursts of four stimuli (100 Hz), inter-burst interval of 200 msec for 30 s) was used; post-tetanus values were recorded for 1 h.

For data analysis, I/O curves of population spike amplitude and latency were compared for pre-baseline values from all groups (two-way analysis of variance (ANOVA), treatment × stimulus). Latency analysis (stimulus artefact to peak) was limited to signals > 0.5 mV (i.e. for stimulus intensities ≥15 V), since smaller signals do not allow reliable measurements.

LTP time courses were calculated relative to baseline values (in percentage) for both population spike amplitude and latency. Data are presented as mean ± standard error of means (S.E.M). For multiple comparisons of LTP and I/O data, two-way repeated measure ANOVA with post hoc tests (Bonferroni) were conducted for all post-tetanus values. For time point analysis at 1, 10, 30 and 60 min post-tetanus, one-way ANOVA followed by unpaired *t*-tests were used. Significance was determined as $P < 0.05$ (significant), $P < 0.01$ (highly significant).

Overall, HCY injected rats appeared healthy and undistinguishable from water-exposed controls. They did not show any signs of distress or altered behaviour in their home cage. In comparison with controls, no significant