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High sensitivity of cerebellar neurons to homocysteine is determined by expression of GluN2C and GluN2D subunits of NMDA receptors

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ABSTRACT

Homocysteine (HCY) induced neurotoxicity largely depends on interaction of this endogenous amino acid with glutamate NMDA receptors (NMDARs). This receptor type is composed by GluN1 and different GluN2 (A, B, C or D) subunits. However, the receptor activity of HCY in brain regions which differ in relative contribution of GluN2 subunits was not tested so far.

In the current study, we explored the action of HCY on cerebellar neurons which natively express GluN2C and GluN2D subunits of NMDARs and compared this with the action of HCY on cortical neurons which are mainly composed by GluN2A and GluN2B subunits. To validate obtained results, we also studied the responses to HCY in recombinant GluN1/2C and GluN1/2D NMDARs expressed in HEK293T cells. Responses to HCY were compared to membrane currents evoked by glutamate or by the specific agonist NMDA.

First, we found that on HEK cells expressing GluN1/2C or GluN1/2D NMDARs, HCY was full agonist producing membrane currents similar in amplitude to currents induced by glutamate. The EC_{50} values for these particular receptor subtype activation were 80 μM and 31 μM, respectively. Then, we found that HCY similarly to NMDA, evoked large slightly desensitizing membrane currents in native NMDARs of cerebellar and cortical neurons. In cortical neurons, the ratio of the respective currents (I_{HCY}/I_{NMDA}) was 0.16 and did not significantly change during *in vitro* maturation. In sharp contrast, in cerebellar neurons, the ratio of currents evoked by HCY and NMDA was dramatically increased from 0.31 to 0.72 from 7 to 21 day in culture. We show that least 75% of HCY-induced currents in cerebellum were mediated by GluN2C- or GluN2D-containing NMDARs. Thus, our data revealed a large population of cerebellar NMDA receptors highly sensitive to HCY which suggest potential vulnerability of this brain region to pathological conditions associated with enhanced levels of this neurotoxic amino acid.

1. Introduction

Hyperhomocysteinemia, an elevated level of endogenous amino acid L-homocysteine (HCY) in blood and cerebrospinal fluid, represents a risk factor for stroke [1] and can contribute to severe neuronal disorders including neurodegenerative ones such as Parkinson's [2,3] and Alzheimer's diseases [4]. Blood plasma HCY composition can reach over 100 μM [5,6]. We previously showed that the high level of HCY led to the neuronal cell death through activation of NMDA receptors (NMDARs) and mGluR5 [7]. Plenty of evidence is accumulating showing that HCY is an agonist operating via the glutamate binding sites of NMDARs [8–10] and can differently modulate NMDAR desensitization depending on GluN2 subunit compositions [10]. We, in addition, demonstrated recently that GluN2A subunit-containing NMDARs represent the preferential targets for HCY and can contribute to neuronal pathology through activation by HCY of synaptic GluN1/2A receptors, whereas GluN2B subunit-containing NMDARs are desensitized by this amino acid [11]. In general, there is a consensus that neuronal NMDARs represent an important target for the action of elevated HCY [8,9,11–13]. Nevertheless, the specific features, associated with the action of HCY on NMDARs composed of GluN2C and GluN2D subunits are still missing.

It has been shown that cerebellum as a brain structure that is

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