Do Stereoisomers of Homocysteic Acid Exhibit Different Convulsant Action in Immature Rats?

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Received March 21, 2019
Accepted September 25, 2019

Summary

Mechanism of ictogenesis of D- and L-stereroisomers of homocysteic acid was studied in 12-day-old rats by means of antagonists of N-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. There was no qualitative difference between the two stereoisomers in generation of emprosthotonic (flexion) as well as generalized tonic-clonic seizures. Moderate differences were observed in the first, nonconvulsive effects of the two isomers. As generation of the two types of seizures is concerned, NMDA and AMPA participate in generalized tonic-clonic seizures whereas NMDA receptors play a dominant role in generation of flexion seizures.

Key words

Homocysteic acid • Seizures • Immature rats • NMDA antagonists • AMPA antagonists

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Introduction

Excitatory amino acid glutamate mediates communication between nerve cells as a ligand to three types of ionotropic receptors – AMPA, NMDA and kainate (Wisden and Seeburg 1993). Different role of first two types of receptors is generally recognized – AMPA receptors serve for fast communication between neurons, NMDA receptors are deeply involved in potentiation phenomena and memory (Meldrum 2000). Both AMPA and NMDA receptors are overexpressed during postnatal development with a peak around the end of the second postnatal week (Erdö and Wolff 1990, Insel *et al.* 1990, Brennan *et al.* 1997, Arai *et al.* 1997). In addition to glutamate, sulphur containing amino acids might participate in excitatory transmission. Homocysteic acid (HCA), a potent excitatory and convulsant agent, is physiologically present in the brain (Olney *et al.* 1987, Cuenod *et al.* 1990). HCA was shown to be an agonist of NMDA type of glutamate receptors (Cuenod *et al.* 1990) present in hippocampus and released by electrical stimulation (Klancnik *et al.* 1992). Presence of HCA was demonstrated in glial cells (Grandes *et al.* 1991).

Majority of data on the action of HCA in the nervous system was generated with a racemate (D/L-HCA) but the two stereoisomers might have different mechanisms of action (effects on NMDA and AMPA receptors) as demonstrated in experiments with intracerebroventricular application of either stereoisomer (Turski 1989). Single neuron studies yielded controversive results – Herrling described differential effects of the two stereoisomers on feline caudate neurons (Herrling *et al.* 1989) whereas Kilic demonstrated the same action of these isomers on cerebellar granule cells (Kilic *et al.* 1992). We tested convulsant action of the two stereoisomers administered intraperitoneally in developing rats where blood-brain barrier is not yet mature (Saunders 1977) and systemic administration of NMDA elicits two types of seizures – age-bound flexion (emprosthotonic) seizures and classical generalized tonic-clonic seizures (Mareš and Velíšek 1992). In addition,

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there is at least a quantitative difference between the two stereoisomers with D-HCA active in lower doses than L-isomer (Mareš *et al.* 1997). We used an action of NMDA and AMPA receptor antagonists against seizures induced by the two stereoisomers of HCA to answer the question if D- and L-HCA have different mechanisms of action.

Methods

All procedures involving animals and their care were conducted according to the ARRIVE guidelines https://www.nc3rs.org.uk/arrive-guidelines in compliance with national (Act No 246/1992 Coll.) and international laws and policies (EU Directive 2010/63/EU for animal experiments) and the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). The experimental protocol was approved by the Ethical Committee of the Institute of Physiology of the Czech Academy of Sciences. Male Wistar rat pups 12 days old were used. Their body temperature was maintained by means of a pad heated electrically on 34 °C (i.e.to the temperature in the nest) during whole experiment. Animals were pretreated by antagonists of excitatory amino acid NMDA receptors CGP40116 (0.1, 0.5 and 5 mg/kg 30 min before HCA) or MK-801 (0.1, 0.5 and 2 mg/kg 30 min before HCA) and/or AMPA receptors NBQX (15 and 30 mg/kg 15 min before HCA) or GYKI 52466 (10 and 20 mg/kg 5 min before HCA). All compounds were dissolved in saline, NBQX should be mildly warmed and adjusted with sodium hydroxide to a slightly alkaline pH (8.0) to completely solubilize this compound. The doses and interval between injection of antagonists and HCA were based on our data with pentylenetetrazol-induced seizures.

Two doses of either stereoisomer of HCA (Tocris) were administered intraperitoneally – 4 and 8 mmol of L-HCA/kg and 1.4 and 4 mmol of D-HCA/kg. Doses are based on our older data demonstrating different susceptibility of 12-day-old rats to the two stereoisomers (Mareš *et al.* 1997). Either stereoisomer was dissolved in saline and pH was corrected to 7, volume of injection was always 1 ml per 100 g of body weight. Each group was formed by eight animals.

Animals were observed in isolation in plastic boxes for 30 min and incidence of two types of seizures (flexion and generalized tonic-clonic) and mortality was registered.

Incidence was statistically compared by means of Fisher test, $p<0.05$ was taken as significant.

Results

In agreement with our older data both stereoisomers were able to elicit flexion as well as generalized tonic-clonic seizures, D-isomer was active at lower doses than L-isomer. As the first signs of HCA action are concerned they were different, administration of L-HCA led to twisting of the tail and hind half of the body in all cases. D-HCA elicited these movements only exceptionally, forelimb shuffling was common. Hyper-locomotion and barrel rolling were observed after both stereoisomers.

Antagonists of NMDA receptors

Either antagonist suppressed frequent tail and body twisting elicited by L-HCA less efficiently than this less frequently appearing phenomenon induced by D-HCA. There was no difference in suppression of other behavioral phenomena appearing early after the two stereoisomers.

The competitive antagonist CGP40116 more effectively suppressed both types of seizures elicited by L-HCA than seizures induced by D-HCA (Fig. 1). The same difference was demonstrated for a noncompetitive antagonist MK-801 only for generalized tonic-clonic seizures (Fig. 2). On the contrary, flexion seizures were abolished only with the highest dose of MK-801 (2 mg/kg). As expected, antagonists were more active against lower doses of either stereoisomer.

Antagonists of AMPA receptors

Tail and body twisting elicited by L-HCA were less effectively suppressed by both NBQX and GYKI 52466 than if elicited by D-HCA. The same difference was observed in barrel rolling but not in other behavioral phenomena.

The competitive antagonist NBQX did not exhibit any significant action on either stereoisomer, only a tendency to suppression of flexion seizures elicited by D-stereoisomer was found (Fig. 3). The noncompetitive antagonist GYKI 52466 suppressed seizures elicited by L-HCA but not those elicited by D-HCA (Fig. 4).

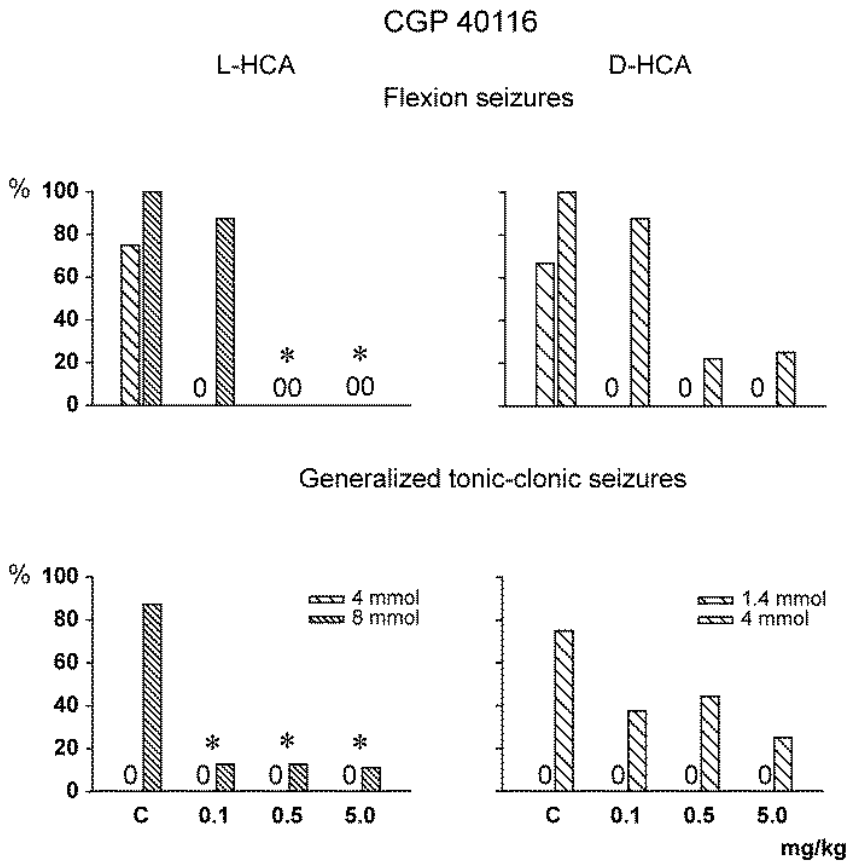

Fig. 1. Effect of CGP 40116 on incidence of seizures elicited of the two stereoisomers (L-HCA on the left, D-HCA on the right). Upper graphs – empro-sthotonic, flexion seizures, lower graphs – generalized tonic-clonic seizures. X-axis: doses of the antagonist, C means control rats injected with solvent; Y-axis: percentage of animals exhibiting seizures. 0 denotes absence of seizures, asterisks significant difference from controls.

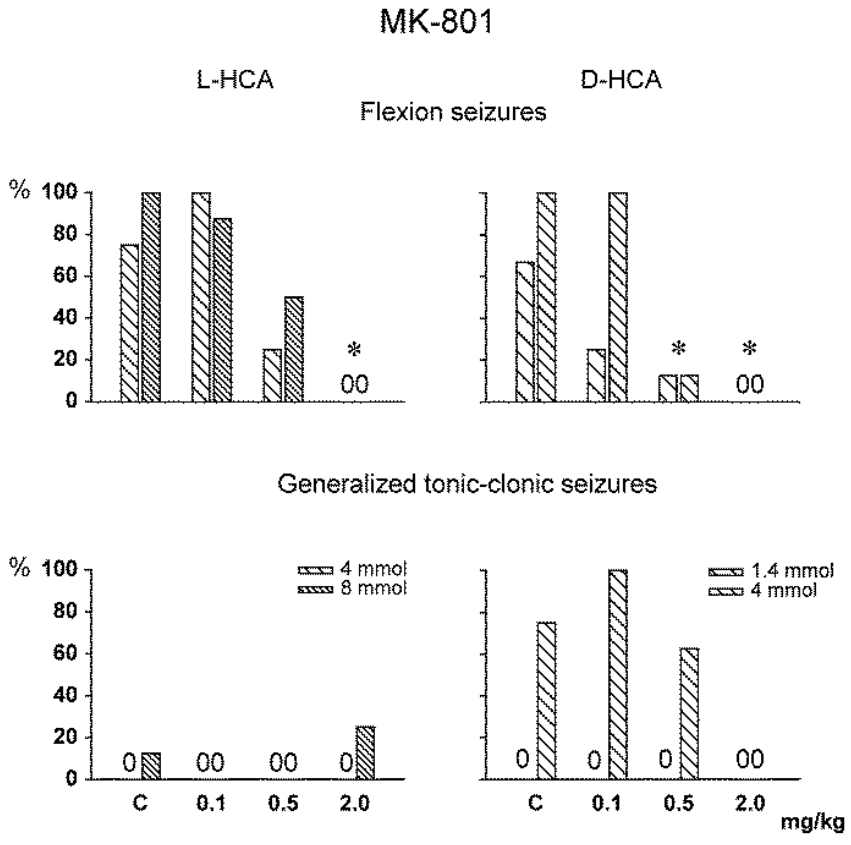

Fig. 2. Effect of MK-801 on incidence of seizures elicited of the two stereoisomers. All details as in Fig. 1.

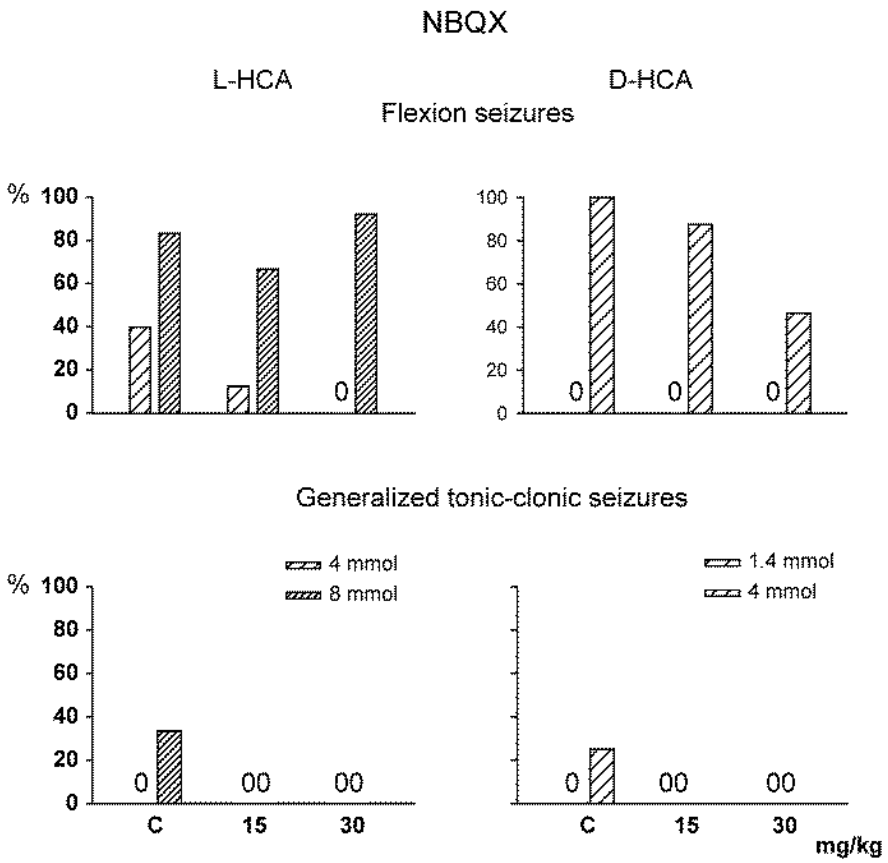

Fig. 3. Effect of NBQX on incidence of seizures elicited of the two stereoisomers. All details as in Fig. 1.

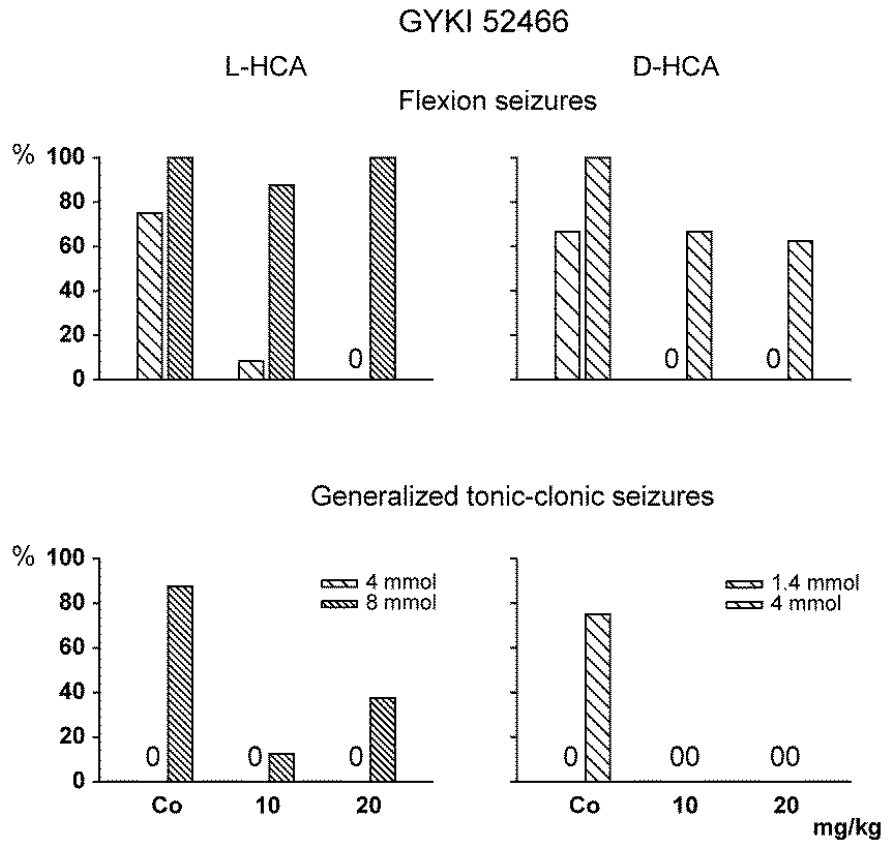

Fig. 4. Effect of GYKI 52466 on incidence of seizures elicited of the two stereoisomers. All details as in Fig. 1.

Discussion

Our present results confirmed differences in first signs of action of the two stereoisomers as well as stronger convulsant action of D-stereoisomer in comparison with L-stereoisomer described by Turski (1989) and in our previous paper (Mareš *et al.* 1997). Our pharmacological results demonstrated reliable action of NMDA receptor antagonists against both types of seizures in contrast to AMPA antagonists where anticonvulsant effects were only moderate if any.

The two types of seizures (flexion and generalized tonic-clonic) are possibly generated in different brain structures by a little different mechanisms. Generalized tonic-clonic seizures are of brain stem origin with participation of more brain structures (Browning and Nelson 1985, Browning and Nelson 1986, Mareš 2006) the probable site of origin of flexion seizures is not known in spite of the fact that this type of seizures is used to model early infancy human seizures (Scantlebury *et al.* 2010, Velisek *et al.* 2007). Both ionotropic excitatory receptor systems are involved in generation of GTCS whereas NMDA is much more important than AMPA in generation of flexion seizures. GTCS are more sensitive to anticonvulsant action of NMDA antagonists than flexion seizures especially when elicited with L-stereo-isomer. Generally, NMDA receptor antagonists are more active against flexion seizures than AMPA antagonists, this difference is not so marked in the case of GTCS.

We cannot explain the difference in suppression of seizures between the two AMPA antagonists NBQX (more active against flexion seizures elicited by D-isomer) and GYKI (more active against either type of seizures elicited by L-isomer). More detailed analysis should be made.

Conclusions

Seizures induced by HCA are sensitive to antagonists of both NMDA and AMPA receptors. GTCS could be more easily affected than flexion (emprosthotonic) seizures. As concerns differences between NMDA and AMPA antagonists, NMDA antagonists are more efficient against seizures elicited by L-HCA (especially flexion seizures) but this difference is only quantitative. The same mechanism of ictogenesis with a dominant role of NMDA receptors is used by both stereoisomers.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

This study was funded by grant No. 18-09296S of the Grant Agency of the Czech Republic and by a project PHARMABRAIN, reg. No. CZ_02.1.01/0.0/0.0/16_025/0007444.

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