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The Time-dependent Block of NMDA Glutamate Receptor Influences Hippocampal LTP in Inborn Cerebellar Degeneration Mouse Model

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Abstract: The effect of single dose of NMDA glutamate receptor blockage administration on the hippocampal LTP was evaluated in animal model of inborn cerebellar degeneration. We compared the level of possible LTP blockade in two groups of animals, Lurcher mutant mice and their healthy littermates which served as controls. In the second part of the study we tested group of mice which were influenced repeatedly by the same NMDA blocker (MK-801) during behavioral experiments. Our results suggest a similar effect of blockade either after single or chronic MK-801 administration; both of them practically disrupted LTP generation with differences between healthy and neurodegenerative animals.

Introduction

N-methyl-D-aspartate receptors (NMDAR) are members of the ionotropic glutamate receptor family. They mediate the excitatory synaptic transmission in the mammalian central nervous system (CNS). These receptors reveal some special properties, as activity of glycine, which acts like co-agonist, a relatively high calcium (Ca^{2+}) permeability and important voltage-sensitive block of ion channel permeability by extracellular magnesium (Mg^{2+}). In case of resting membrane potential, the NMDAR remains inactive, and only depolarizing stimulus can activate it [1]. After activation, a relatively slow-rising and long-lasting current develops. The physiological function of NMDAR was undoubtedly confirmed for neuronal survival, differentiation and synaptic plasticity. An increase of NMDAR activity also leads to long-term changes in neuronal functions including protein kinase cascades as well as modulation of transcription factors [2]; these changes suggest that NMDAR function is especially important in mechanisms of learning and memory on the cellular level [3].

This work is focused on the modulation of hippocampal long-term potentiation (LTP) after blockade of NMDA receptors by MK-801. LTP was discovered as a result of repetitive high-frequency electrical stimulation (tetanus) of one pathway with the increase of the excitatory synaptic potentials to a subsequent single pulse. This phenomenon represents a generally accepted model of memory at the synaptic level [4]. MK-801 (dizocilpine) is a highly potent, selective and non-competitive NMDA receptor antagonist which acts as an open channel blocker [5]. Design of experimental procedures consisted of two parts:

1st – a study of the possible effect of short-term NMDAR blockade of the hippocampal LTP in Lurcher mutant mice (LMM) and their healthy controls – wild – type mice (WT).

2nd – an evaluation of possible long-lasting interaction between cerebellar and hippocampal activity in LMM in which NMDAR were repeatedly influenced and experimental animals were investigated using three different kinds of learning and memory tests before LTP procedure.

Experimental Material and Methods

Our experimental animal model (LMM) represents a natural kind of genetically determined olivocerebellar degeneration. The primary etiological factor is a mutation in the delta2-glutamate receptor gene expressed preferably by the Purkinje cells [6]. Delta receptors (GluRδ) were classified as special kind of ionotropic glutamate receptors but their properties and function are not yet fully understood. Recent experimental results suggest that GluRδ are localized in the cerebellum, auditory and vestibular system [7]. Heterozygote individuals of LMM (+/Lc) are characterized by postnatal complete loss of cerebellar Purkinje cells where mechanisms of excitotoxic apoptosis were confirmed. Decreased number of granule cells and inferior olivary neurons is secondary as a consequence of the loss of Purkinje cells. Affected homozygotes (Lc/Lc) are not viable. Unaffected homozygotes (WT) – wild type mice (+/+) are completely healthy and serve as controls.

In the first part of present study, adult Lurcher mutant mice (n=12) and wild type (n=18), (C3H strain of both sexes, bred in our laboratory), were used. All experiments were performed in full agreement with the EU Guidelines for scientific experimentation on animals and with the permission of the Ethical Commission of the Faculty of Medicine in Pilsen.

Hippocampal LTP was performed as an acute experiment under urethane anaesthesia (20%, 1,5 g/kg b.w., intraperitoneally). After the loss of nociceptive and corneal reflexes animal was fixed into the stereotaxic frame. Body temperature was measured by rectal probe and small heating pad (Fine Science Tools, USA) was used for temperature keeping (37 °C ± 0,5). Then a surgical preparation and calva cleaning were done. Using high-speed micro-drill (Fine Science Tools, USA) the corresponding holes were prepared; for stimulation in perforant path: **(AP – λ, L – 3.0, V – 2.0)** and registration in ipsilateral hilus of dentate gyrus **(AP – 2.0, L – 1.7, V – 1.9)** stainless steel electrodes were used. Grounding electrode was fixed with the screw to the bone in the contralateral prefrontal area. All calculation has been done according to the bregma point [8]. For the basal low frequency (LFS), 16 biphasic pulses 2 – 4 V, 0.1 Hz, duration 0.1 ms, for high-frequency stimulation (HFS) 100 Hz, 3 bursts each 15 s were applied.

Experimental protocol consisted of four parts:

1st – registration of basal response (then used as average value from 3 responses after LFS – 100%);
2nd – MK-801 administration in dose 0.15 mg /1kg b.w., 3 min before HFS;
3rd – high-frequency (tetanic) stimulation (HFS);
4th – registration of responses after HFS – time intervals 5th, 10th, 15th, 20th, 30th, 45th and 60th min.

The final statistical evaluation was performed by the ANOVA test using the population spike amplitude as a comparable parameter.

In the second part of study experimental animals (C3H strain of both sexes, WT – n=9, LMM – n=8) after long-term behavioral studies with MK-801 were used. These tests were performed during three intervals of postnatal ontogeny (D15–D16, D29–D31, D36–D40) and consisted of three methods (briefly); dose of MK-801 was 0.1 mg/kg b.w. 15 min. before (in each day of experimental session!).

Test of inhibitory learning – passive avoidance ("step-down", PA-SD; D15–D16
In the PA-SD paradigm the animals were positioned on the safe bench located above the electrified grid which allowed mice to stay, but not to move on the bench. The criterion was to stay 100 second on the safe bench without descent [9].

Tests of motor learning; D29–D31
Motor coordination was investigated with a set of three tests: horizontal bar, ladder and rotarod [10]. All tests were repeated four times in one run. The criterion of successful trial was to stay on the apparatus for 60 s or to leave it actively.

Spatial navigation test; D36–D40
The Morris water maze method [11] was used for examination of spatial learning. Four trials a day were performed consecutively from 4 different starting points. Animals were placed into a circular pool (diameter 95 cm) filled with water. Their task was to find a round platform, which was hidden about 0.5 cm under the water surface, and to climb up it. If the mouse did not reach the platform within 60 s, it was placed there. The mouse stayed on the platform for 30 s. Final evaluation was the comparison of meeting criteria to reach the platform within 60 with the percentage of trials on individual days and mean escape latencies.

After 1 week – lasting pause, animals were used in the LTP experimental procedure, in the same paradigm as described above.

Results

Our results confirmed the ability of LTP maintenance in both kinds of unaffected animals (i.e. WT and LMM) with greater average of amplitude (Figure 1) in healthy mice. MK-801 pre-treatment caused a clear decrease of magnitude of population spike amplitudes, in both groups. The period of effective NMDA receptor blockade was not permanent (Figure 2); in WT mice it lasted from 5th to 30th minute. LMM revealed lower level of the blockade but with prolongation to the 60th minute. Both healthy and neurodefective mice revealed similar level of the LTP blockade whereas clear statistically significant difference between controls and affected animals was described (Figure 3).

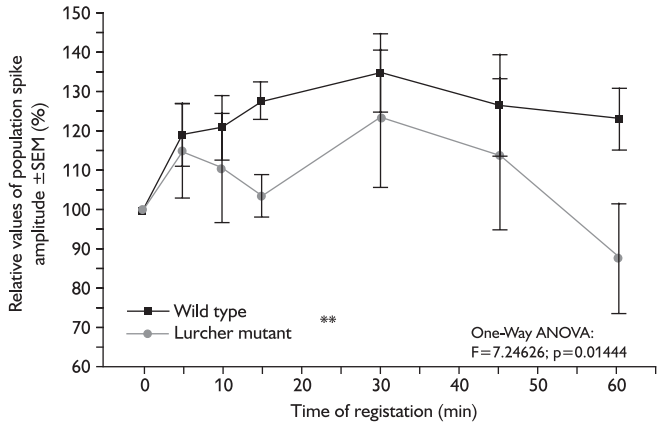

Figure 1 – Comparison of relative values of the amplitude (in %) ± SEM in unaffected wild-type (squares) and Lurcher mutant mice (circles).

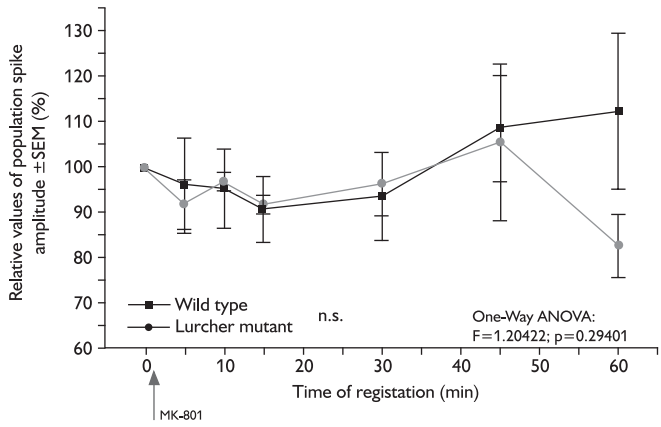

Figure 2 – Comparison of relative values of the amplitude size (in %) ± SEM in wild-type animals (squares) and Lurcher mutant mice (circles) after a single dose of MK-801.

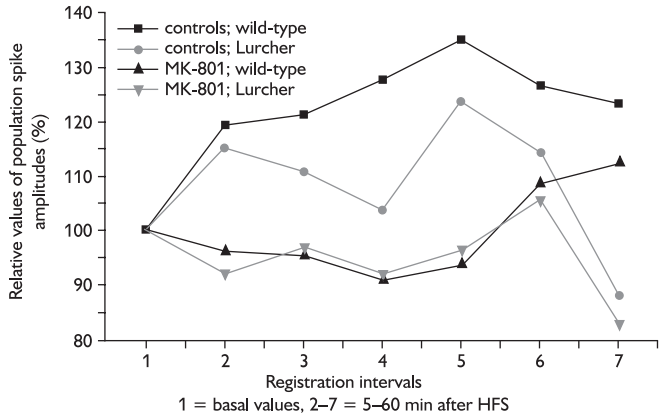

Figure 3 – Summarising view on the magnitude of population spike amplitude (in %) in both groups of mice; unaffected (squares and circles) and with MK-801 pre-treatment (triangles).

Analysis of LTP protocol after long-term NMDAR blockade showed permanent decrease of amplitude values (Figure 4) or practical inability to create LTP. Differences between WT animals and LMM were not statistically significant.

Discussion

In recent studies in rodents, NMDA receptor (NMDAR) blockade impaired learning and memory by disruption of LTP, especially spatial navigation which is hippocampal-dependent activity [12]. Some studies in humans suggest that blocking NMDAR impairs encoding (ie. mechanism of learning), but not retrieval (i.e. memory); similar results were shown both in spatial and nonspatial information processes [13, 14]. From previous studies we know that LTP mechanism includes two main parts. First is the postsynaptic depolarization mostly via activation of AMPA and/or kainate channels which allows opening of NMDA channels. Ca^{2+} influx increases and the higher intraneuronal concentration of Ca^{2+} has several secondary effects [3, 15]. The administration of MK-801 blocks this possible secondary enzymatic activation like calmodulinkinases, proteinkinases and nitric-oxide synthases pathways. Second part of LTP induction is localized at the presynaptic level. The retrograde messengers (nitric oxide, arachidonic acid and probably CO) are formed as a result of Ca^{2+} increase after NMDA receptor activation [16]. These messengers (or neuromodulators) reach the presynaptic nerve terminal by diffusion, where they enhance transmitter releasing into the synaptic cleft. We suggest also effect of MK-801 at the presynaptic level [17].

The results of our previous experiments which studied the development of spatial learning in LMM and WT during the first month of life using the standard Morris water maze showed that some cognitive functions in LMM are changed [18]. Some brain neurons in Lurchers are more sensitive to neurotoxic substances [19] and they display a higher degree of excitability of the CNS in LMM when compared with WT [20]. Similar findings we obtained in examination of brain cortical activity after previous electrical and drug

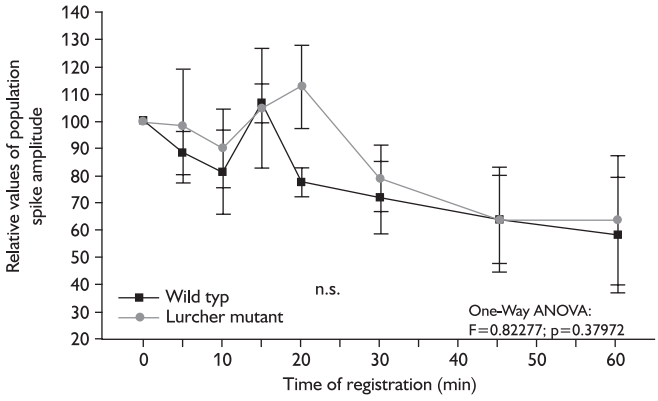

Figure 4 – Comparison of relative values of amplitude size (in %) ± SEM in mice influenced by the MK-801 during long-term behavioral tests; wild-type (squares) and Lurcher mutant mice (circles).

stimulation [21, 22]. Also significant changes of hippocampal activity (LTP) were found in anesthetized neurodegenerative mice in comparison with their healthy littermates [23, 24].

Our study demonstrates that primary cerebellar pathology, i.e. mutation in the GluRδ gene may have a possible secondary influence on the level of NMDAR blockade caused by MK-801. We expect (and also speculate from our recent results), that the ability of the higher LTP production on one side and higher spatial navigation ability on the other are not identical [25]. These findings support the idea that mechanisms of spatial learning (i.e. the hippocampal LTP at synaptic level) involve a number of nonspatial components (motor control or stress factors) that do not depend on the hippocampus and can influence final results [26].

Conclusions

NMDAR blockade (both in acute experiment and after long-term MK-801 administration) caused similar changes in animals of both groups (i.e. LMM and WT) and disrupted of LTP production and maintenance. We expect a close cooperation between brain structures which are involved in mechanisms of learning and memory, i.e. between the cerebellar and hippocampal neuronal circuits.

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