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Deletion of neuronal gap junction protein connexin 36 impairs hippocampal LTP

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Abstract

In the mammalian CNS, deletion of neuronal gap junction protein, connexin 36 (Cx36), causes deficiencies in learning and memory. Here we tested whether Cx36 deletion affects the hippocampal long-term potentiation (LTP), which is considered as a cellular model of learning and memory mechanisms. We report that in acute slices of the hippocampal CA1 area, LTP is reduced in Cx36 knockout mice as compared to wild-type mice. Western blot analysis of NMDA receptor subunits indicates a higher NR2A/NR2B ratio in Cx36 knockout mice, indicating that there is shift in the threshold for LTP induction in knockout animals. Data suggest a possibility that learning and memory deficiencies in Cx36 knockout mice are due to deficiencies in LTP mechanisms.

Keywords

Gap junctions; connexin 36; LTP; hippocampus; NMDA receptors

Introduction

In the mammalian central nervous system (CNS), direct intercellular communication between neighboring cells occurs through electrical synapses (gap junctions). A neuron-specific gap junction protein, connexin 36 (Cx36), is expressed in many CNS regions, including the hippocampus [2, 7]. Knockouts for Cx36 were generated and revealed a number of deficiencies, including reduced synchronization between interneurons in the cortex, deficiency in visual transmission and impaired cerebellar motor learning (reviewed in [21]). In addition, the high-frequency network oscillations (ripples), γ-oscillations and θ-rhythm in the hippocampus, that are putative correlates of memory engram inscription, are reduced in Cx36 knockout mice or after pharmacological blockade of gap junctions [3, 4, 14, 16]. Finally, spatial learning, object memory and fear learning and memory, that are believed to be dependent on the hippocampal circuitry, are impaired in Cx36-deficient mice [3, 10]. These data suggest the role for Cx36-containing gap junctions in a number of CNS functions, including learning and memory.

Long-term potentiation (LTP) of chemical synaptic transmission in the mammalian hippocampus is considered as a cellular model of learning and memory mechanisms [17].

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LTP also can be induced in mixed chemical and electrical synapses on the Mauthner cells of goldfish and the activity of chemical synapses affects LTP in electrical synapses, suggesting interaction of chemical and electrical synaptic transmission systems [5, 20]. In the mammalian CNS, the expression of neuronal gap junctions is regulated by neurotransmitter receptors [18], supporting the interaction of chemical and electrical synapses. However, whether neuronal gap junctions affect LTP in chemical synapses was not known. In the present study we tested the hypothesis that deletion of neuronal Cx36 impairs LTP in the hippocampal CA1 area.

Materials and methods

The use of animal subjects in these experiments was approved by the University of Kansas Medical Center Animal Care and Use Committee. The experiments were performed on 2 months old wild-type (WT; C57bl/6 strain) and Cx36 knockout (C57bl/6 background strain) male mice in accordance with NIH guidelines. The Cx36 knockout was originally created by Dr. David Paul (Harvard Medical School). Mice were genotyped as described [9].

Acute brain slices were prepared and perfused using the media as described in details [1]. Evoked field excitatory postsynaptic potentials (fEPSP) were induced and measured using electrical stimulation and extracellular recording as described previously [1]. A recording electrode was placed in the stratum radiatum of the CA1hippocampal region and stimulating electrode was located in the area containing Schaffer collaterals. For analysis of fEPSPs, a 30 min background recording was conducted using low-frequency stimulation (0.033 Hz; 0.05 ms impulse duration) and the adjusted intensity that induced fEPSPs with ~40% of the maximal fEPSP amplitude. LTP was induced using the same stimulus duration and intensity, but a high frequency stimulation (HFS; 100 impulses, 100 Hz). After HFS, the electrical stimulation was continued using the initial (background) stimulus parameters. The slope of fEPSPs was determined and analyzed as described [1]. The tests were conducted blindly to the genotype of the experimental animal.

Western blot experiments were conducted using the approaches and antibodies as described in detail [23]. Band optical density was determined using Quantity One software (Bio-Rad, USA). Optical density signals were normalized relative to tubulin and normalized values were compared to controls (set at 1.0). Tubulin levels per unit of total protein did not vary significantly among samples.

Data were analyzed using the two-tailed Student's *t*-test or one-way ordinary ANOVA with post hoc Tukey and InStat software (GraphPad Software, USA). Data are reported as mean ± SE for the number of samples indicated.

Results

HFS of the Schaffer collaterals induced rapid and significant increase in the slope of fEPSPs in the hippocampal CA1 region in slices obtained from WT mice (Fig.1a,b). However, there was no significant increase in the fEPSP slope in Cx36 knockout animals (Fig.1a,b). In addition, the normalized fEPSP slope in post-HFS conditions was statistically different between the WT and Cx36 knockout mice (Fig.1b).

It has been suggested previously that a diminished synaptic inhibition results in the increased excitatory synaptic activity, which then leads to increase in the NR2A/NR2B NMDA receptor subunit ratio and occlusion of LTP [25]. Therefore, we tested whether a reduction in LTP in Cx36 knockout mice is associated with increase in the NR2A/NR2B ratio. Western blots revealed that the expression of NR2A subunit was significantly higher in Cx36 knockout than in WT mice, however, no difference in the expression of NR2B

subunit between the two animal groups was observed (Fig.2a,b). As a result, the NR2A/NR2B ratio was higher in Cx36 knockout than in WT animals (Fig.2c).

Discussion

Previous studies demonstrated that LTP can be induced in mixed (electrical and chemical) synaptic contacts on the goldfish Mauthner cell and that the LTP in electrical synapses is modulated by changes in chemical synaptic activity [5, 20]. In the present study, we show that chemical synaptic LTP in the mouse hippocampus is altered in the absence of neuronal gap junction protein Cx36. This suggests the role for neuronal gap junctions in the mechanisms of chemical synaptic LTP.

A reduction in the mouse hippocampal LTP by a non-specific gap junction blocker, carbenoxolone, has been reported previously [6]. It must be noted, however, that carbenoxolone blocks not only neuronal gap junctions, but also non-neuronal gap junctions and hemichannels. Moreover, it has multiple non-junctional side effects [19, 22]. In the present research, we used a Cx36 knockout mouse that represents selective elimination of neuronal gap junction protein and, therefore, is more specific.

We found that the NR2A/NR2B ratio is elevated in the hippocampus of Cx36 knockout mice. It has been suggested [15, 24, 25] that increase in the NR2A/NR2B ratio may result in the increase in threshold for LTP induction. Therefore, the increase in the ratio may be a potential mechanism for down-regulation of the hippocampal LTP in Cx36-deficient animals. Further, it has been demonstrated [12], that interneurons may contribute to the induction of hippocampal LTP. In the CNS, subpopulations of interneurons are interconnected via Cx36-containing gap junctions, and this local network controls the activities of pyramidal neurons [11, 13]. In addition, the frequency of inhibitory postsynaptic currents is significantly diminished in Cx36 knockout mice [8], suggesting reduction in inhibitory neurotransmission. Therefore, another possible mechanism for down-regulation of the hippocampal LTP in Cx36-deficient mice is elimination of gap junctions between interneurons.

Our study shows that the expression of NR2A receptor subunit is increased in the hippocampus of Cx36 knockout mice. A focus of future studies will be to determine how elimination of neuronal gap junction protein changes the expression of a chemical neurotransmitter receptor. Further, it will be important to determine whether the NR2A up-regulation is due to elimination of neuronal gap junctions or whether it is simply due to deletion of Cx36 protein and is unrelated to decrease in the coupling.

As discussed above, a number of studies show deficiencies in learning and memory in mice lacking neuronal gap junction protein, Cx36. LTP of synaptic transmission in the hippocampus is considered as a cellular model of learning and memory mechanisms [17]. Our study shows that the hippocampal LTP is dramatically reduced in mice lacking Cx36. Thus, the data suggest a possibility that learning and memory deficiencies in Cx36 knockout mice are due to deficiencies in the mechanisms of LTP.

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Research Highlights

> Genetic deletion of neuronal Cx36 causes learning and memory deficiencies.

> Here we tested whether genetic deletion of Cx36 affects hippocampal LTP.

> We find that LTP is reduced in Cx36 knockout mice.

> Analysis shows a higher NR2A/NR2B receptor subunit ratio in Cx36 knockout mice.

> Reduced LTP may be the cause of learning and memory deficits in Cx36 knockout mice.

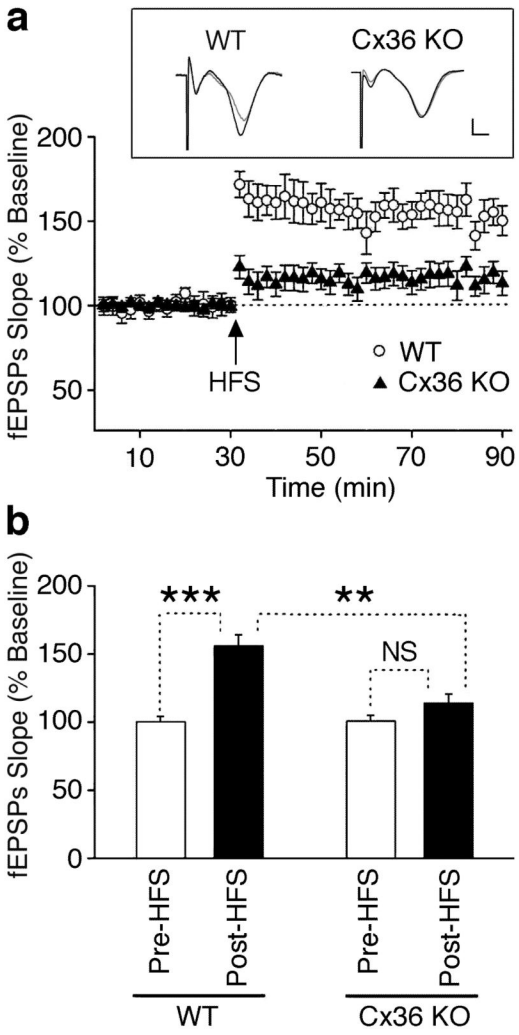

Fig.1.

LTP does not occur in the hippocampal CA1 region of Cx36 knockout mice. Averaged traces of normalized (relative to baseline) fEPSP slopes (**a**) and statistical analysis (**b**) are shown. In **a**, the time of HFS is indicated by a vertical arrow. In **b**, the pre-HFS (baseline) data are from 10-min-long background recordings obtained immediately before HFS; the post-HFS data are from recordings obtained between 50 and 60 min after HFS. In both panels, data are shown as mean ± SE; n = 5-6. Statistical analysis: one-way ordinary ANOVA with post hoc Tukey; ***P<0.001; **P<0.01; NS, non-significant. Insert in **a**: fEPSPs from pre-HFS recordings (gray) and from recordings obtained between 50 and 60 min post-HFS (black); left panel, WT; right panel, Cx36 knockout (Cx36 KO); vertical bar, 0.2 mV; horizontal bar, 1 ms.

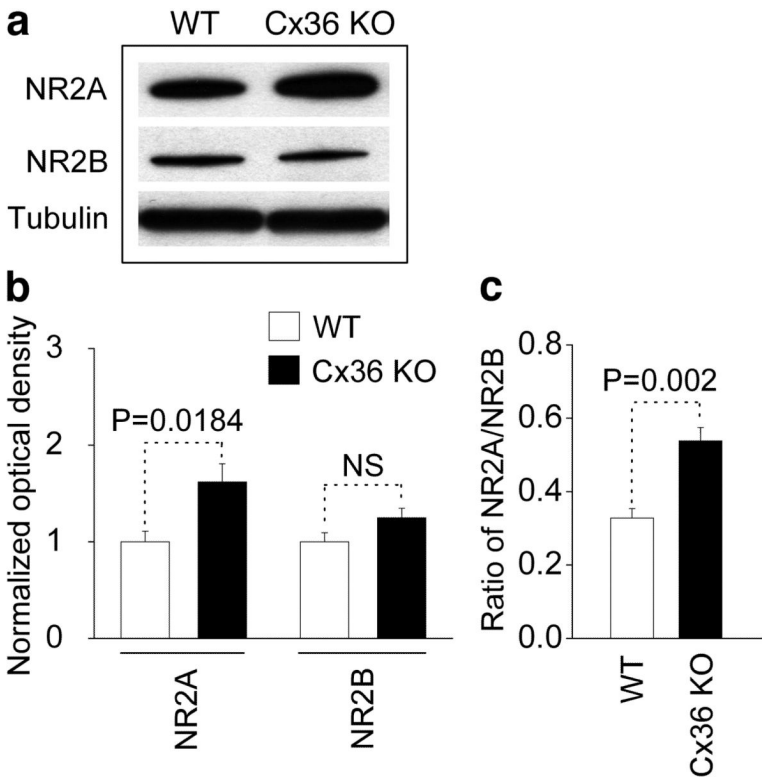

Fig.2.

The NR2A/NR2B ratio is higher in the hippocampus of Cx36 knockout mice than in WT mice. Data from western blot experiments are shown. **a,b,** Representative image (**a**) and statistical analysis (**b**) of the expression of NR2A and NR2B receptor subunits are shown. (**c**) Graph presents the analysis of the NR2A/NR2B ratio. Statistical analysis: paired (**b**) and unpaired (**c**) Students *t*-test; $n = 6\text{-}7$; mean ± SE. Optical density signals are normalized relative to tubulin. The westerns were done sequentially on one membrane.