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Neuronal serine racemase regulates extracellular D-serine levels in the adult mouse hippocampus

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Abstract

In the hippocampus of mice lacking the gene for serine racemase (SR), a D-serine synthesizing enzyme, in the CaMKII α -expressing neurons, we observed a significant decrease in the extracellular concentration of D-serine, a coagonist for the N-methyl-D-aspartate type glutamate receptor (NMDAR), and NMDAR hypofunction as revealed by diminished extracellular taurine concentrations after an intra-hippocampal NMDA infusion when compared to the wild type controls. Therefore, the neuronal SR could regulate the extracellular D-serine signaling responsible for NMDAR activation in the hippocampus.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The present experiments were performed in accordance with the guidance of the Tokyo Medical and Dental University and were approved by the Animal Investigation Committee of the Institution.

Keywords

CaMKII α -expressing neurons; Hippocampus; In vivo microdialysis; *N*-methyl-D-aspartate type glutamate receptor; D-Serine; Serine racemase

Introduction

It is widely accepted that D-serine acts as an endogenous coagonist for the *N*-methyl-D-aspartate subtype glutamate receptor (NMDAR) by stimulating its glycine modulatory site. Thus, selective elimination of D-serine by D-amino acid oxidase or D-serine deaminase in the forebrain tissues has been reported to result in marked attenuation of the NMDAR function in vivo and in vitro in rodents (Balu and Coyle 2015; Nishikawa 2011; Wolosker 2007). The functional connection between D-serine and NMDAR is further supported by the neuroanatomical observation that the distribution pattern of the tissue and extracellular contents of D-serine in the brain is closely correlated with the NMDAR density (Balu and Coyle 2015; Nishikawa 2011; Wolosker 2007). In fact, various NMDAR-mediated biochemical, electrophysiological, cellular and behavioral consequences are markedly disturbed by genetic elimination of the D-serine synthesizing enzyme, serine racemase (SR), which reduces the tissue and extracellular D-serine contents to approximately 10 % of those of the wild type mice (Balu and Coyle 2015; Nishikawa 2011; Wolosker 2007).

Recently, Benneyworth et al. (2012) demonstrated an impaired hippocampal NMDAR function in mice lacking SR gene in the forebrain CaMKII α -expressing neurons, which use glutamate as their neurotransmitter. However, NMDAR function was normal in mice in which serine racemase gene was inactivated only in the brain astrocytes. The hippocampal tissue contents of D-serine in the neuronal SR knockout mice was not reduced, suggesting that NMDAR dysfunction was attributed to a diminished concentration of the extracellular D-serine. To test this hypothesis, we studied the extracellular D-serine levels measured by in vivo dialysis in the hippocampus of mice deficient in the SR gene in the CaMKII α -expressing neurons. We have also examined the effects of an intra-hippocampal infusion of NMDA on the extracellular taurine content to evaluate NMDAR function.

Materials and methods

Animals

The present experiments were performed in accordance with the guidance of the Tokyo Medical and Dental University and were approved by the Animal Investigation Committee of the Institution. Neuron-specific SR gene deficient mice (nSR $-/-$) were generated as previously described (Benneyworth et al. 2012). These mice are homozygous for the floxed (fl) SR construct (Basu et al. 2009) and heterozygous for the Ca²⁺/calmodulin-dependent kinase II α (CaMKII Cre2834) that produces the Cre expression only in the forebrain glutamatergic neurons beginning at postnatal day 17 and reaching near adult levels by day 34 (Schweizer et al. 2003). All genetic constructs used in this experiment were maintained on a C57BL/6 J mouse background (backcrossed 10 generations). The control mice used in this experiment were the SR fl/fl genotype that did not carry the Cre transgene. We used the

nSR^{-/-} and nSR^{+/+} (control) mice after postnatal weeks 12, weighing 25–35 g, because the SR expression and D-serine concentration maximally decreased after this age (Benneyworth et al. 2012).

In vivo microdialysis

The in vivo microdialysis was performed as previously depicted with some modifications (Ishiwata et al. 2013a, b). A straight cellulose dialysis tube (1.0 mm in length, 0.16 mm internal diameter, molecular weight cutoff 50,000, EICOM Co., Ltd., Japan) was implanted into the hippocampus (A -2.2 mm, V 1.5 mm, L 1.3 mm) according to the atlas of Paxinos and Franklin (2004) on a stereotaxic frame under pentobarbital anesthesia (40 mg/kg, intraperitoneally). Two days after surgery, the dialysis probe was perfused with a Ringer solution (NaCl, 147 mM; KCl, 4 mM; CaCl₂, 1.3 mM; pH 7.4) at a flow rate of 2 µl/min in the freely moving mouse. After stabilizing for at least 80 min, the dialysate samples were collected every 20 min. The first five samples were used to determine the basal level of each amino acid, and then the experiment was started by the fifth sampling of the dialysate designated as time 0. A stimulation of the NMDAR was achieved by perfusing the Ringer solution containing 150 µM NMDA from time 0 to 10 min in the hippocampus. The collected dialysates were stored at -80 °C until use, following the addition of D-homocysteic acid as the internal standard.

Tissue preparation

Mice were killed by cervical dislocation. The hippocampus was rapidly removed on the ice, frozen in liquid nitrogen, and stored at -80 °C. The brain sample was homogenized in 10 volumes of 4 % trichloroacetic acid with D-homocysteic acid, and the supernatant resulting from centrifugation of the homogenate at 14,500g for 20 min at 4 °C was stored at -80 °C until derivatization.

Biochemical assay and data analysis

We derivatized the dialysates or supernatants with *N*-tertbutyloxycarbonyl-L-cystein and *o*-phthalaldehyde for 2 min at room temperature and quantified various amino acids by HPLC with fluorometric detection as mentioned before (Ishiwata et al. 2013b).

We performed statistical comparisons using the unpaired two-tailed Aspin–Welch's *t* test between the control and SR deficient groups on the extracellular and tissue concentration data of the various quantified amino acids during the basal period at each experimental time point.

Results

The deletion of SR gene in the CaMKII-positive neurons resulted in a significant decline in the extracellular D-serine concentration as compared to controls in the hippocampus (-43 %: Fig. 1a; Table 1). The extracellular concentrations of L-glutamate, an NMDAR agonist, of L-serine, a precursor for the D-serine biosynthesis, and of glycine, another NMDAR coagonist, were unchanged in the brain region (Table 1; Fig. 1c, d). Furthermore, the extracellular taurine and alanine levels of nSR^{-/-} mice were significantly reduced in the hippocampus

(Fig. 1b) to approximately 74 and 73 %, respectively, of the control values. The absolute basal concentration values of various amino acids determined in the hippocampus in this study are summarized in Table 1.

A 10-min intra-hippocampal infusion of NMDA at 150 μ M caused a significant increase in the hippocampal extracellular taurine concentrations of the control (Fig. 1e circle) and nSR^{-/-} (Fig. 1e, square) mice. The percentages of the maximal NMDA-induced increase in the taurine concentration as compared to the basal levels are similar between the control (Fig. 1e, 146 %) and nSR^{-/-} (Fig. 1e, 152 %) mice. Therefore, the maximal absolute concentrations of the extracellular taurine following the NMDA infusion in the nSR^{-/-} mice were still lower than in the control mice (μ M): control, 4.944 ± 0.452 , $n = 7$; nSR^{-/-}, $3.047 \pm 0.382^{**}$, $n = 11$; $^{**}P < 0.01$ (Fig. 1e).

In the hippocampus of the nSR^{-/-} mice, there was a significant reduction in the tissue contents of D-serine (Table 1). The magnitude of the reduction is similar to that seen in our previous experiment (Benneyworth et al. 2012) although the difference in the tissue levels of D-serine between the wild type and nSR^{-/-} mice was not statistically significant possibly due to the relatively small number of mice in the previous study. The SR gene deficit produced a significant diminution or increase in the tissue contents of L-glutamate (-3 %) or alanine (+29 %), respectively, without affecting those of taurine, L-serine, glycine, L-glutamine and L-threonine in the hippocampus (Table 1).

Discussion

In the present study, we demonstrated using an in vivo microdialysis technique that the nSR^{-/-} mice exhibited significantly lower basal contents of the hippocampal extracellular D-serine and taurine than the littermate nSR^{+/+} control mice. The dialysate concentrations of taurine after NMDA infusion, as an index of NMDAR function, remain diminished in a similar magnitude in the hippocampus of the nSR^{-/-} mice when compared to the littermate controls.

These observations suggest the hypoactivity of the hippocampal NMDAR in the nSR^{-/-} mice that determined the lesser extracellular release of taurine, because it has been shown through in vivo and in vitro experiments that the extracellular taurine contents are under a phasic facilitatory regulation by the NMDAR in the rodent hippocampus (Lehmann et al. 1985; Menendez et al. 1989; Rodriguez-Navarro et al. 2009; Shibasaki et al. 1993). The diminished taurine levels following NMDAR stimulation in the hippocampal dialysates as shown in Fig. 1e are in line with the suppression of the NMDAR-mediated component of the EPSC and of the NMDAR-dependent LTP that were previously observed in the hippocampal slices prepared from our SR deficit mice (Benneyworth et al. 2012).

The hippocampal NMDAR dysfunction reflected by these indices could be elicited by lowered extracellular levels of endogenous agonists for the glutamate or glycine site of the glutamate receptor, which include glutamate, or glycine and D-serine, respectively (Nishikawa 2011). Significant reduction in the basal extracellular concentrations of D-serine, but not glutamate and glycine, favors the ideas that the decreased extracellular D-serine

signal is likely to contribute, at least in part, to the NMDAR hypofunction. The chronic deficit in the NMDA receptor function produced by diminution in the extracellular D-serine levels could result in the reduction of the basal extracellular taurine release as seen in the present study, while an acute interruption of the NMDAR-mediated transmission fails to alter the extracellular taurine concentrations in the hippocampus.

The exact molecular pathways underlying the decreasing effects of the CaMKII α -positive neuron-specific attenuation of the SR expression on the extracellular D-serine contents are still unclear. The simultaneous decrease in the tissue and basal extracellular levels of D-serine (Table 1) indicates that an amount of D-serine in its releasable pool might depend on that the storage pool of D-serine synthesized by neuronal SR or that D-serine could be liberated to the extracellular space directly from the store site. This phenomenon also suggest that SR is a crucial member of a retain system for the extracellular D-serine concentration that is essential for physiological activation of the NMDAR.

It is also interesting to note that the conditional SRKO caused a much greater reduction in the extracellular levels of D-serine than in the tissue levels. This discrepancy could be explained by the presence of non-CaMKII α -expressing neurons and/or non-neuronal cells that synthesize D-serine by SR. It is also possible that D-serine could be provided by another pathway other than the racemization of L-serine, e.g., D-serine in food, undefined synthesis processes, etc. The total SRKO has indeed been reported to cause an incomplete loss of the tissue D-serine concentrations to approximately 10 % of the wild type controls (Balu and Coyle 2015; Nishikawa 2011).

The disturbed D-serine synthesis in the CaMKII α -expressing neurons could be related to changes in the tissue and/or extracellular levels of L-glutamate and L-alanine. Because L-alanine has been suggested to facilitate the extracellular D-serine release through Asc-1 by a heteroexchange mechanism (Maucler et al. 2013; Rosenberg et al. 2013), the reduced extracellular and tissue levels of D-serine could be accompanied by a change in the efflux and tissue accumulation of L-alanine in the hippocampus. The slight, but significant, decrease in the hippocampal tissue levels of L-glutamate would be accounted for by a compensatory increase in the efflux of L-glutamate from the cells for the reduced NMDA receptor activity produced by the drop in the extracellular D-serine concentrations. This hypothesis appears to be consistent with the present observation that the extracellular L-glutamate concentrations tended to elevate in the hippocampus (Table 1).

In conclusion, our data extrapolate the view that SR in the CaMKII containing neurons regulates the extracellular D-serine signaling to the NMDAR in the hippocampus in vivo. Further investigations are needed to clarify the molecular machinery of release of D-serine to the synaptic cleft and the precise mechanisms bridging between the SR and the machinery.

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Fig. 1.

Extracellular concentrations of D-serine, taurine, L-serine and glycine in the hippocampus of the nSR^{-/-} and nSR^{+/+} mice. **a-d** Basal extracellular contents of D-serine, taurine, L-serine and glycine in the hippocampus (**a-d**, respectively) of the nSR^{-/-} and nSR^{+/+} (control) mice are determined by averaging the values of each amino acid of consecutive 5 time points after an 80-min stabilization. The results are expressed by means with SEM of data obtained from 7 to 11 animals per each group: * $P < 0.05$ compared to the control group; *NS* no significant difference. **e** Effects of a 10-min infusion of NMDA (150 μM) on the

extracellular taurine contents in the hippocampus of the nSR^{-/-} and nSR^{+/+} (control) mice. Each point represents the mean with SEM of data obtained from 7 to 11 animals. There was a statistically significant difference ($P < 0.01$) in the values between nSR^{-/-} and nSR^{+/+} groups at 40 min

Table 1

Tissue and basal extracellular concentrations of various amino acids in the hippocampus of the nSR^{-/-} and nSR^{+/+} mice

Amino acids	Extracellular contents (μM)		Tissue contents (nmol/mg tissue)			
			% of Control (%)	Hippocampus		% of Control (%)
	Hippocampus					
	nSR+/+	nSR-/-		nSR+/+	nSR-/-	
D-Serine	0.21 ± 0.03 (9)	0.12 ± 0.01 (12)**	57	0.27 ± 0.01 (15)	0.24 ± 0.01 (19)*	89
Taurine	3.26 ± 0.26 (8)	2.42 ± 0.20 (12)*	74	10.20 ± 0.37 (15)	10.28 ± 0.29 (19)	101
L-Serine	1.16 ± 0.18 (9)	0.86 ± 0.07 (12)	74	1.17 ± 0.19 (15)	1.27 ± 0.16 (19)	109
Glycine	1.92 ± 0.16 (8)	1.51 ± 0.18 (13)	79	1.36 ± 0.08 (15)	1.40 ± 0.08 (19)	103
L-Glutamate	0.91 ± 0.13 (8)	1.07 ± 0.20 (12)	118	11.35 ± 0.14 (15)	10.96 ± 0.11 (19)*	97
L-Glutamine	8.86 ± 1.06 (9)	6.69 ± 0.80 (12)	76	7.47 ± 0.30 (15)	8.28 ± 0.62 (19)	111
L-Threonine	0.84 ± 0.08 (7)	0.75 ± 0.07 (12)	89	0.35 ± 0.05 (15)	0.34 ± 0.04 (19)	97
L-Alanine	1.41 ± 0.09 (8)	1.03 ± 0.09 (12)*	73	1.14 ± 0.08 (15)	1.43 ± 0.07 (19)*	125
GABA	ND	ND		4.79 ± 0.11 (15)	4.67 ± 0.08 (19)	95

In the present experiments, nSR^{+/+} mice are used as littermate controls. The concentrations of various amino acids are not corrected for recovery from each microdialysis probe. The number of animals is shown in parentheses

GABA γ-amino butylic acid, ND not determined

* $P < 0.05$ or

** $P < 0.01$ compared to the control group