The Role of Lysyl Oxidase-Like Protein (LOXL) in Mouse Hippocampal Long-Term Potentiation

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Abstract

Lysyl oxidase-like protein (LOXL) is a member of the LOX copper-dependent amine oxidase family and plays a role in the development and maintenance of the extracellular matrix. Immunohistological staining (rabbit polyclonal antibody 1:500) of transverse hippocampal slices showed LOXL was expressed in the dentate gyrus of LOXL+/+ but not LOXL-/- mice, and not in the CA1 pyramidal cell layer of either. Dentate granule cells from LOXL-/- mice were smaller than those in LOXL +/+ mice. We tested the hypothesis that lack of LOXL and altered morphology may result in changes in neuronal plasticity in the dentate gyrus. Electrical recordings were obtained in 300 μm hippocampal slices in the dentate (following perforant path stimulation) and in the CA1 pyramidal cell layer (following stimulation of the stratum radiatum). Potentiation (measured 30 minutes after tetanus) was expressed as a percentage of the pre-tetanus population spike amplitude. Potentiation in the CA1 cell layer of 12 LOXL-/- and 12 LOXL+/+ mice was 188.4 ± 9.3% and 184.4 ±13.7%, respectively (mean ±SE). The dentate showed potentiation of 119.2 ±7.0% and 119.3 ±3.9% in 12 LOXL-/- and 12 LOXL+/+ mice, respectively. As measured by PSA there was no significant difference in potentiation between LOXL+/+ and LOXL-/- mice in either the dentate granule or CA1 pyramidal cell layers. Because there are marked dentate structural changes in LOXL-/- mice, we conclude that in LOXL-/- mice other structural proteins may compensate so that pre- and post-synaptic elements remain functionally positioned to ensure viable synaptic plasticity.

Introduction

For over a century the medial temporal region of the brain, and more specifically the hippocampus, has been the suspected site of long-term memory disorders such as amnesia. However, until 1986 there had not been any published clinical reports in which the human subjects had damage to the hippocampus alone. Because of this absence, understanding of the nature of the link between memory and the hippocampus was based on speculation.

On September 18, 1978 a patient named R. B. suffered an ischemic episode that resulted from an arterial tear following coronary bypass surgery. During this episode R. B. developed a bilateral lesion in the CA1 region of his hippocampus but no other significant damage elsewhere in the brain. Zola-Morgan[1] reported that this lesion resulted in an anterograde amnesia in which R. B. could remember everything he learned prior to the surgery but could not recall events that occurred afterward. This finding strongly supported the speculation that the hippocampus plays an important role in memory and learning.

The hippocampus may facilitate the storage of information by enabling increases in synaptic efficiency for very specific neural circuits. Bliss[2,3] confirmed this possibility when in the early 1970s he reported that high frequency stimulation of specific neural pathways in the hippocampus led to an increase in synaptic strength. This increase is now termed "Long-Term Potentiation" or LTP. While LTP is not permanent, it does last a matter of hours in vitro and days in vivo[5] and may contribute to the conversion of short-term to long-term memory[4].

The cellular mechanism responsible for LTP induction involves the activation of two post-synaptic glutamate receptors, NMDAR and AMPAR, by a high frequency stimulus termed tetanus. During single shock stimulation, the pre-synaptic terminal releases moderate levels of glutamate. This neurotransmitter binds to AMPAR on the post-synaptic membrane to open a channel so that ions may flow down their concentration gradient into the cell, moderately depolarizing the post-synaptic membrane. NMDA receptors lie dormant, their channels plugged by Mg^{++} ions. During tetanus, high levels of glutamate are released and the post-synaptic membrane is strongly depolarized to knock the Mg^{2+} plug off of the NMDAR and allow its channel to open. Ca^{2+} may now enter the post-synaptic cell through the NMDA channel and induce a cascade of biochemical reactions that in turn induce the up-regulation of post-synaptic receptors, causing an increase in the excitability of the post-synaptic cell. At this point low frequency stimuli evoke a much larger response than previously in the hippocampal network, the hallmark of LTP (Fig. 1).

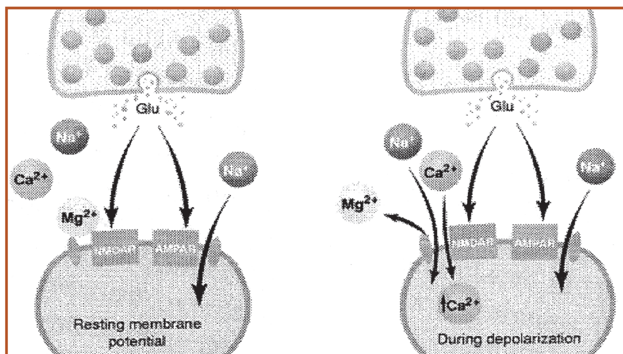

Figure 1. The cellular components of LTP. Note that increased glutamate release (right) activates the NMDAR and allows calcium to flow into the post-synaptic cell, an event that does not occur during normal transmission but that sets off a biochemical cascade that serves to establish and maintain LTP. (Figure courtesy of Nicoll and Malenka.[5])

The proteins collagen and elastin are cross-linked by the enzyme lysyl oxidase to form a rigid matrix that supports cellular structures and membrane components. Lysyl oxidases comprise a family of copper-dependent amine oxidases that link collagen and elastin fibers of the extracellular matrix by oxidizing and binding inter- and intra-molecular peptidyl lysines.[6,7] The pre-and post-synaptic endings of hippocampal dentate granule cells may be held in alignment by this extracellular matrix. The AMPAR and NMDAR, two post-synaptic glutamate receptors critical to the development of LTP, may also be held in place by the proteins collagen and elastin of the extracellular matrix.

Recently, researchers at the University of Hawaii developed a knockout mouse that does

not express lysyl oxidase-like protein (LOXL), and found that dentate granule cells in the hippocampus of this animal were smaller than those observed in the hippocampus of wild-type littermates. A mouse deficient in the LOXL enzyme may not be able to properly align critical synaptic components and thus may not be able to generate LTP.

Hence, we hypothesized that lack of LOXL and the resulting altered morphology of dentate granule cells may result in altered neuronal plasticity in the dentate gyrus.

Materials and Methods

ANIMALS

Twenty-four mice of approximately eight weeks of age were obtained from the University of Hawaii and housed according to standards approved by the UC Davis Animal Care and Use Committee. All mice were housed together in a 12:12 light:dark cycle and fed *ad libitum*. Experimental animals consisted of four groups: 6 male LOXL-/-, 6 female LOXL-/-, 6 male LOXL +/+, and 6 female +/+. All animals were of a C57BL6 genetic background and, to ensure objectivity, experimenters who performed slice experiments were kept unaware of the subject's genotype at the time of experiments.

TISSUE PREPARATION AND INCUBATION

Each animal was sacrificed between 0800 and 0840 PST by decapitation during the first hour of its light cycle. The brain was immediately excised and placed in high sucrose/low sodium artificial cerebrospinal fluid (HSLSaCSF) for one minute at approximately 3°C. The hippocampus was removed and then sectioned to 300µm thick slices using a vibratome. Hippocampal slices were immediately placed in HSLSaCSF at approximately 33.8 ˚C and perfused with 95/5% O_2/CO_2 gas for 30 minutes, then allowed to cool passively to room temperature over a period of 30 minutes. Slices were subsequently transferred via wide mouth pipette to standard aCSF perfused with 95/5% O_2/CO_2 gas, where they remained until the time of experiment.

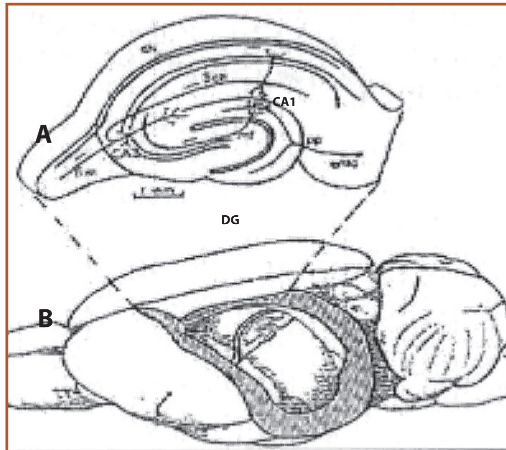

Figure 2. The anatomy of the hippocampus. A schematic diagram showing the hippocamus in the brain (B), the hippocampal slice, and the lamellar organization of the hippocampal neural network (A). Note the pathway between the dentate gyrus (DG) and the CA1 pyramidal cell layer.

LTP INDUCTION

Electrodes were placed in the slice in two geometrical arrangements: 1) to record evoked responses in the dentate gyrus, tungsten stimulating elctrodes were placed in the perforant pathway (pp) and a borosilicate glass recording electrode was placed in the dentate gyrus granule cell layer (DG); 2) to record evoked responses in the CA1 pyramidal cell layer, tungsten stimulating electrodes were placed among the Schaffer collaterals (SCA) in the CA3 region of the stratum radiatum and a borosilicate glass recording electrode was placed in the CA1 pyramidal cell layer (Fig. 2).

The protocol to determine whether an evoked population response was enhanced by tetanus was as follows:

1) Evoked responses for increasing stimulus amplitude were recorded in order to construct an input/output curve so that we could establish the half-maximal stimulus. Then a series of four averages of five evoked responses each was recorded over 20

minutes to establish a baseline response at a half-maximal stimulus.

2) The slice was tetanized—shocked with a half-maximal voltage at 100Hz for one second five times.
3) A series of seven averages of five evoked responses each was recorded over thirty minutes to establish a post-tetanus response at half-maximal stimulus.
4) The mean of the last four responses was divided by the mean of the four baseline responses, and expressed as a percent to determine the degree of potentiation.
5) An evoked response was recorded at a stimulus intensity greater than half-maximal to ensure that the slice was not saturated.

Results

LTP was reliably established and maintained (Fig. 3), but there was no statistically significant difference between mice of different genders or genotypes (Fig. 4). Potentiation in the CA1 cell layer of 12 LOXL-/- and 12 LOXL+/+ mice was 188.4 ±9.3% and 184.4 ±13.7%, respectively (mean ±SE). The dentate showed potentiation of 119.2 ±7.0% and 119.3 ±3.9% in 12 LOXL-/- and 12 LOXL+/+ mice, respectively. LTP was consistently higher when measured in the CA1 region of both genotypes and genders, relative to the LTP measured in the dentate gyrus region of the hippocampus (Fig. 5).

As indicated by immunohistochemical staining, LOXL was found to be expressed by the dentate granule cells in the hippocampus of LOXL+/+ mice, but not of LOXL+/- or LOXL-/- mice. LOXL was not expressed in the CA1 region of any mice (Fig. 6).

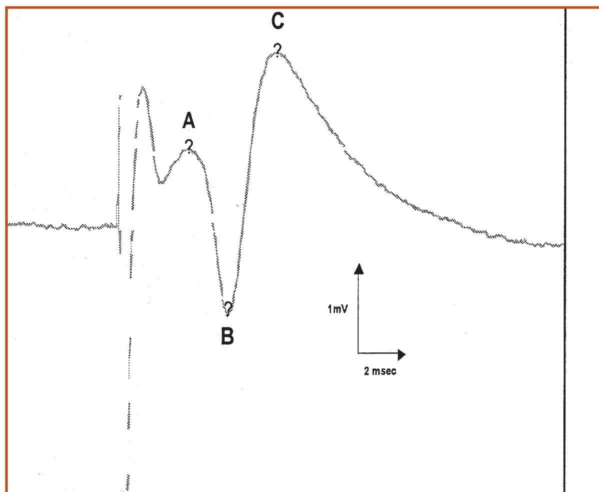

Figure 3. Population spike amplitude. The amplitude of the evoked response was determined by using the equation [(A-B)+(C-B)]/2.

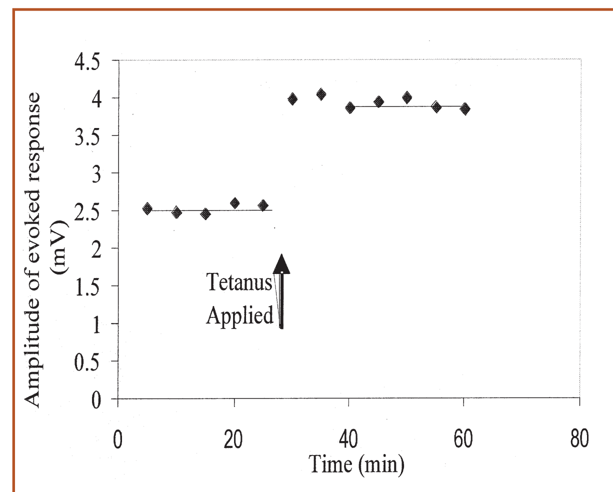

Figure 4. A representative recording of LTP in the hippocampus. A stable baseline was recorded over 20 minutes, a tetanus was applied, and recordings were taken at the same half-maximal stimulus intensity for 35 minutes after tetanus. The evoked response increases dramatically and remains increased after a tetanus is applied. This long-lasting increase is indicative of LTP.

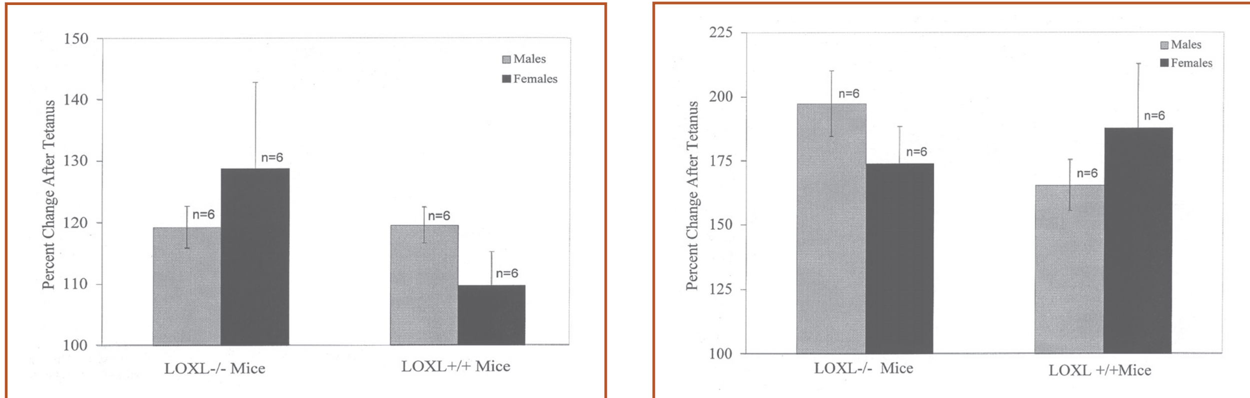

Figure 5. The LTP of mice as measured by population spike amplitude, grouped by genotype and gender. A) LTP measured in the CA1 region of the hippocampus. B) LTP measured in the dentate gyrus region of the hippocampus. Means are expressed ± standard error.

Figure 6. Selected localization of LOXL in the hippocampus of LOXL+/+, -/-, and +/- mice. The presence of lysyl oxidase-like protein is shown as a light rust-colored immunohistochemical stain while blue represents nuclear staining. A) Lysyl oxidase is expressed in CA3 (bottom right) but not in CA1 (top left) pyramidal cells of the wild-type mouse. B) LOXL is expressed in the dentate gyrus of LOXL+/+ mice but not in that of LOXL +/- or -/- mice. C) LOXL is expressed in the denate granule cells of LOXL +/+ mice but not in those of LOXL +/- or LOXL-/- mice. LOXL is not expressed in the CA1 pyramidal cells of any of these animals. (Photomicrographs courtesy of Dr. Pingan Li and Dr. Katalin Csiszar, University of Hawaii.)

Discussion

The current study suggests that plastic changes, and more specifically LTP, in the dentate granular and CA1 pyramidal circuits of the hippocampus are not dependant on the presence of lysyl oxidase-like protein. While structural differences were observed between the dentate granule cells of LOXL-/- mice and those of LOXL+/+ mice, the ability to establish and maintain LTP was equal in both groups. Accordingly, while LOXL may be needed for the proper development of dentate granular cells, such development is not needed in either the establishment or maintenance of LTP. However, one alternative explanation for this finding may be that developmental mechanisms have compensated for structural deficiencies resulting from the lack of LOXL. For example, other proteins in the lysyl oxidase family which serve a similar function as LOXL may have been up-regulated in response to the LOXL deficiency imposed by our knockout. As a result, any effects of the knockout may be masked by compensatory mechanisms. Further studies of the expression of other lysyl oxidases in this model must be completed to rule out such a possibility.

Acknowledgments

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