氏　　名　WU Yue

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学 位 論 文 題 目　Target-cell-specific Left-Right Asymmetry of NMDA Receptor Content in Schaffer Collateral Synapses in e1 Knock-out Mice

論 文 審 査 委 員　主　査　教授　伊佐　正
教授　川口　泰雄
教授　Peter Somogyi（Oxford 大学）

論文内容の要旨

N-methyl-D-aspartate (NMDA) receptors mediate excitatory neurotransmission and activity-dependent changes in synaptic efficacy in the central nervous system and play an important role in learning and memory. NMDA receptors are composed of seven known subunits- GluRζ1 (NR1), GluRε1-4 (NR2A-2D) and GluRχ1-2 (NR3A-3B), and functional activities of the NMDA receptor channel require the heteromeric assemblies of obligatory GluRζ1 with one or more other subunits.

In the hippocampal CA1 area, pyramidal cells and GABAergic interneurons receive excitatory inputs from Schaffer collaterals (Sch) arising from the ipsilateral CA3 pyramidal neurons and commissural fibers (com) arising from the contralateral CA3 pyramidal neurons (Ishizuka et al., 1990). The fast excitatory synaptic transmission from these inputs is mostly mediated by NMDA and AMPA type glutamate receptors. The NMDA receptors in the Sch and com fiber synapses on pyramidal cells contain ζ1, ε1, and ε2 subunits in adult rodents (Monyer et al., 1994; Fritschy et al., 1998; Takumi et al., 1999; Racca et al., 2000). Some interneurons in the hippocampus also express ε4 subunit as well as ζ1, ε1, and ε2 subunits (Monyer et al., 1994; Standaert et al., 1996; Standaert et al., 1999).

The asymmetrical allocation of NMDA receptor ε2 (NR2B) subunits was discovered in the Sch-CA1 pyramidal cell synapses between the left and right hippocampus and between the apical and basal dendrites of single neurons (Kawakami et al., 2003). Direction of this asymmetry depends on inputs; com-pyramidal cell synapses have a mirror-image asymmetry to that for Sch-pyramidal cell synapses (Kawakami et al., 2003). Although electrophysiological and morphological studies have suggested differential localization of glutamate receptors depending on target-cell types as well as on input pathways (Shigemoto et al., 1996; Nusser et al., 1998b; Gottmann et al., 1997; Ito et al., 2000), it is not clear whether the asymmetry in ε2 allocation is also related to the types of the postsynaptic cells. In the present study, to examine the asymmetrical ε2 distribution in distinct postsynaptic target cells, I utilized quantitative postembedding immunogold labeling method in the left and right CA1 areas. I used ε1 knock-out (KO) mice to facilitate the detection of difference in the ε2 immunoparticle density.

In naïve ε1 KO mice, I found no significant difference in labeling density for ε2 in pyramidal cell synapses, which are made by both Schaffer collateral and commissural fibers. However, in ε1 KO mice operated for ventral hippocampal commissure transection (VHCT) to examine Schaffer collateral synapses selectively, labeling density for ε2 but not ζ1 and GluR2/3 in Sch-CA1 pyramidal cell synapses was significantly different ($P<0.05$) between the left and right hippocampus. The ratio of ε2 labeling density in the left to right was about 1:1.5 in the stratum oriens and about 1.6: 1 in the stratum radiatum. Moreover, labeling density for ε2 in Sch-CA1 pyramidal cell synapses was significantly different ($P<0.05$) between basal and apical dendrites. The ratio of ε2 labeling density in the basal to apical dendrites was about 1:1.4 in the left hippocampus and about 1.5:1 in the right hippocampus. This result is consistent with the asymmetry in ε2 allocation previously detected with electrophysiology and immunoblot analysis

in wild type mice. On the other hand, the ε2 labeling density was not significantly different (P>0.05) in interneuron synapses between the left and right. Interneurons were grouped into GluR4-immunopositive and GluR4-immunonegative subpopulations in the stratum radiatum. Double immunofluorescence results showed that 88.7% of GluR4-immunopositive interneurons were palvalbumin immunoreactive and 11.1% of GluR4-immunopositive interneurons were mGluR1α immunoreactive. None of GluR4-immunopositive interneurons showed immunoreactivity for calretinin or calbindin. In addition, immunoreactivity for GluR4 was not colocalized with that for ε4 in the CA1 area, indicating that NMDA receptors in GluR4-immunopositive interneuron synapses are composed of ζ1 and ε2 similar to those in the pyramidal cell synapses. The density ratio of ε2 labeling in the left to right was 0.88:1 in Sch-GluR4 immunopositive interneuron synapses and 1.14: 1 in Sch-GluR4 immunonegative interneuron synapses.

Consistent with the anatomical asymmetry in the ε2 distribution in Sch-CA1 pyramidal cell synapses, amplitude of evoked NMDA EPSCs relative to that of non-NMDA EPSCs was different between the left and right CA1 area in VHCT- operated ε1 KO mice. The amplitude ratio of AP5-sensitive EPSCs to DNQX-sensitive EPSCs was larger in the right than left stratum oriens (left, 21.6%±2.64, n = 5 from 5 animals; right, 40.2%±3.40, n = 5, from 5 animals; P<0.01, t-test,). By contrast, the ratio in the stratum radiatum showed a mirror-image asymmetry to that found in the stratum oriens (left, 39.3%±3.40, n = 6, from 6 animals; right, 16.4%±3.11, n = 5, from 5 animals; P<0.01, t-test). Moreover, the asymmetrical ε2 content was directly reflected in different amplitudes of long-term potentiation (LTP) in the left and right stratum radiatum in VHCT-operated ε1 KO mice: in the left Sch-CA1 synapses, the amplitude of LTP was higher than that in the right Sch-CA1 synapses (left, 138%±2.76, n =7, from 7 animals; right, 104% ± 2.77, n = 6, from 6 animals, P <0.05, t-test).

The present results indicate that the target-cell-specific left-right asymmetry of ε2 distribution results in the left-right difference in NMDA receptor content in Sch-CA1 pyramidal cell synapses in ε1 KO mice.

論文審査結果の要旨

中枢神経系の主要な興奮性伝達物質であるグルタミン酸のイオンチャンネル型受容体は速いシナプス伝達に関わるAMPA型受容体と比較的遅い伝達に関わるNMDA型受容体に分類される。最近、川上ら(2003)により、海馬においてCA3領域由来のSchaffer側枝がCA1錐体細胞のapical dendriteとbasal dendriteとの間に形成するシナプスにおけるNMDA型受容体のε2 (NR2B)サブユニットの分布に左右差があることが報告された。この非対称性は入力に依存的で、交叉性線維にはこのような非対称性は見られない。しかし、このようなシナプスにおけるε2 (NR2B)サブユニットの左右非対称的な発現がシナプス後細胞の種類に依存するかどうかは明らかでない。そこで、今回申請者らは、定量的なpostembedding免疫電子顕微鏡法を用いて、左右のCA1領域におけるε2 (NR2B)サブユニットの分布を解析した。特にε2サブユニットの金粒子によるラベルの検出を容易にするためにε1サブユニットのノックアウト(KO)マウスを使用した。

その結果、正常なε1 KOマウスでは錐体細胞のSchaffer側枝及び交叉線維のシナプスにおいてε2サブユニットの分布に左右差はなかったが、交叉線維を切断したε1 KOマウスでは、apical及びbasal dendriteでのε2サブユニットの密度に左右差が見られた。しかしζ1サブユニットやGluR2/3サブユニットの分布には左右差は見られなかった。すなわち、stratum oriensにおいては左右の比は1：1.5であったのに対して、stratum radiatumにおいては左右の比は1.6：1であった。

それに対してε2サブユニットの分布はGluR4サブユニットの発現によって同定される介在ニューロンにおいては左右差は見られなかった。従って左右非対称なε2サブユニットの分布は錐体細胞に特異的であることが明らかになった。

上記のように解剖学的に明らかになった非対称性と同様に、交叉線維を切断したε1 KOマウスにおいて錐体細胞においてwhole cell記録によって記録される興奮性シナプス電流(EPSC)のNMDA成分とnon-NMDA成分の振幅比にも左右差が見られた。特にstratum oriensにおいてこの比は右側で大きく、stratum radiatumにおいてこの比は左側で大きかった。以上の結果はSchaffer側枝—CA1錐体細胞間のシナプスにおけるNMDA受容体ε2サブユニットの分布の左右非対称性が標的細胞特異的であるという新しく興味深い知見を明らかにしたものであり、本論文は学位論文として十分にふさわしい質を有する内容であると審査委員会の委員全員一致で判断した。