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学位論文題目

Involvement of cyclooxynegase-2 in lipopolysaccharide-induced impairment of the newborn cell survival in the adult mouse detate gyrus
（シクロオキシゲナーゼ-2を介したリポ多糖による成体マウス歯状回における細胞新生抑制機構）

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論 文 内 容 要 旨

Recent studies have revealed that the neural stem cells (NSCs), which possess the ability of proliferation and differentiation into neurons and glial cells, exist in the mammalian adult brain, including the anterior subventricular zone (aSVZ) and the hippocampal dentate gyrus. The NSCs in the dentate gyrus are known to lie along the border between the hilus and the granule cell layer (GCL), so called subgranular zone (SGZ), and to migrate into the GCL and to differentiate into the glial cells and the granule cells, resulting in the generation of several thousands of newborn cells each day. Furthermore, the small portion of newly generated neurons is reported to be integrated into existing neuronal circuitries. The role of neurogenesis in the dentate gyrus is still unclear, however, several lines of evidence suggest its involvement in the learning and memory and neuropsychiatric disorders. Interestingly, the proliferation and differentiation of the NSCs as well as the survival of newborn cells are dynamically influenced by a certain type of brain injuries such as ischemia, epilepsy or neuroinflammation. Thus, the identification of the signaling molecules regulating NSC activity may contribute not only to the understanding of the neurogenesis mechanisms but also toward the development of new therapy against neural death.

Recently, lipopolysaccharide (LPS), a bacterial endotoxin, is reported to modulate the neurogenesis in the dentate gyrus of mammalian brain by causing the neuroinflammation including the activation of microglial cells. It has been demonstrated that the peripheral administration of LPS diminishes the neurogenesis in the hippocampal dentate gyrus via the activation of microglial cells in the brain. The neurogenesis consists of the proliferation and the neural differentiation of the NSCs and the survival of newborn cells. However, it is not clear which processes of the neurogenesis are diminished by LPS. Also, the molecular mechanisms underlying the impairment of the neurogenesis elicited by systemic treatment with LPS is not fully understood. In general, the central and the peripheral actions of LPS are mediated or enhanced by the arachidonic acid cascade. In this cascade, two types of cyclooxygenase (COX), COX-1 and COX-2, act as rate-limiting enzymes catalyzing the conversion of arachidonic acid to prostaglandin H_2 and several prostanoids such as prostaglandin E_2 (PGE_2), prostaglandin$F_{2\alpha}$, prostaglandin I_2, prostaglandin D_2 (PGD_2) and thromboxane A_2 are known to be produced from prostaglandin H_2 by specific synthetase. In addition, LPS is known to increase the expression level of COX-2 not only in the peripheral tissues but also in the brain. On the other hand, COX and some prostanoids are reported to involve the modulation of the neurogenesis in the dentate gyrus by the ischemia and the epilepsy. However, little is known about the involvement of COX in the modulatory actions of LPS on the neurogenesis in adult brains. This study was, therefore, designed to determine which processes of the neurogenesis in the adult mouse dentate gyrus are affected by LPS treatment and to clarify the involvement of COX in the LPS actions using the bromodeoxyuridine (BrdU)-pulse chase method and terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) staining.

Firstly, the temporal effect of LPS was assayed at the time point of 2 hrs, 7 and 21 days after its injections. The 2 hrs time point evaluates the proliferation process; 7 and 21 days to differentiation and survival, respectively.

LPS failed to affect the number of BrdU-labeled cells in the dentate gyrus 2 hrs after BrdU injection, indicating no effects of LPS on the proliferation of the NSCs. On the other hand, LPS reduced the number of BrdU-labeled cells in the dentate gyrus 7 days after BrdU injection in a dose-dependent manner. Twenty-one days after BrdU injection, the absolute number of BrdU-labeled cells in the dentate gyrus was lower than that at 7 days after BrdU injection. To confirm whether LPS acts on survival of NSCs, a staining of apoptotic cells was used. The systemic injection of LPS at 1 mg/kg significantly increased the number of TUNEL-positive cells in the dentate gyrus 5 days after the treatment, suggesting that LPS impaired the survival of newborn cells derived from the NSCs.

To investigate which fate of the NSCs could be affected by LPS, the proportion of BrdU-labeled cells co-expressing the immature neuronal marker DCX, the mature neuronal marker NeuN or the astrocyte marker GFAP in the dentate gyrus was examined 7 and 21 days after BrdU injection. We also found that the proportion of cells committed to neural lineage (DCX- and NeuN-positive) and astroglial lineage (GFAP-positive) was not changed by LPS treatment, suggesting that LPS does not affect the differentiation process itself. LPS, therefore, might reduce the survival of immature cells that do not undergo the fate-determination. Alternatively, we cannot rule out the possibility that LPS decreases the survival of neuron-restricted progenitor cells and astrocyte-restricted progenitor cells in the same degree. The NSCs were treated *in vitro* with LPS (0.01, 0.1 and 1 μg/ml) for 24 hrs, and the WST-8 assay was then performed. LPS did not affect the viable cell number of hippocampus-derived NSCs in the presence or absence of EGF, indicating that LPS did not directly affect the proliferation of the NSCs.

The effects of COX inhibitors on LPS-induced impairment of the newborn cell survival were examined in the dentate gyrus. The number of BrdU-labeled cells in the SGZ and the GCL 7 days after BrdU injection was analyzed. The systemic injection of indomethacin (10 mg/kg), a non-selective COX inhibitor, and NS-398 (10 mg/kg), a selective COX-2 inhibitor, completely protected LPS-induced reduction in BrdU-labeled cell number in the dentate gyrus. In contrast, LPS-induced reduction in BrdU-labeled cell number was unaffected by the pretreatment with SC-560 (12 mg/kg), a selective COX-1 inhibitor. Furthermore, the i.c.v. injection of NS-398 (1 μg/10 μl/mouse) also completely protected against LPS-induced decrement in BrdU-labeled cell number.

We investigated the expression of COX-2 in the dentate gyrus of mice with or without LPS treatment. Seven hrs after LPS injection (1 mg/kg), it significantly increased the number of COX-2-positive cells in the GCL and the ML. Even seven days after LPS injection (1 mg/kg), it caused the moderate, but significant increase in the number of COX-2-positive cells in the GCL, but not in the ML. The immunohistochemistry of double-immunostaing for COX-2 and specific cell type markers revealed that the majority of COX-2-positive cells in the GCL co-expressed NeuN and the small portion of COX-2-positive cells in the GCL co-expressed DCX. It was noteworthy that DCX- and COX-2-double positive cells exist only in the GCL, but not in the SGZ. Next, we examined whether the microglia expresses COX-2 in the presence or absence of LPS by staining CD11, which is a maker protein of both the activated and resting microglia. We observed that the number of CD11-positive cells did not alter after LPS treatment, however, its intensity seem to increase by LPS treatment. We found that the majority of microglia did not

express the COX-2 protein in the SGZ, GCL and ML of both control and LPS-treated mice.

To identify the molecular candidates mediating LPS-induced impairment of the newborn cell survival, we utilized the embryonic hippocampus-derived NSCs, which enable us to evaluate the direct actions of drugs. Since PGD_2, 15d-PGJ_2 and PGE_2, are not only known to be produced by COX-2-mediated arachidonic acid pathways and but also reported to show the apoptosis-inducing activity, we tested whether these compounds as well as LPS would cause the cell death using a TUNEL method. PGD_2, 15d-PGJ_2 and PGE_2 increased the cell death of the NSCs in a concentration-dependent manner. Among PGs used in this experiment, 15d-PGJ_2 exhibited the most potent activity inducing cell death in the NSCs. LPS showed a moderate, but significant increment in the cell death only at 1 μg/ml, but not at other concentrations. Taken together, these data suggest that PGE_2, PGD_2, as well as its metabolite 15d-PGJ_2, are feasible candidates mediating the LPS action in the neurogenesis.

In conclusion, the present study confirmed the LPS suppressed the neurogenesis in the adult mouse DG by impairing the newborn cell survival presumably via COX-2-mediated PGD_2, 15d-PGJ_2 and PGE_2 production (Fig. 1). The systemic injection of LPS affects the survival of NSCs due to the activation of COX-2 in LPS-responsive mature granule cells (Fig. 1). These cells release prostaglandins in response to neuroinflammation (Fig. 1). The released PGD_2, 15d-PGJ_2 and PGE_2 promote the cell death, resulting in the suppression of newborn cells survival.

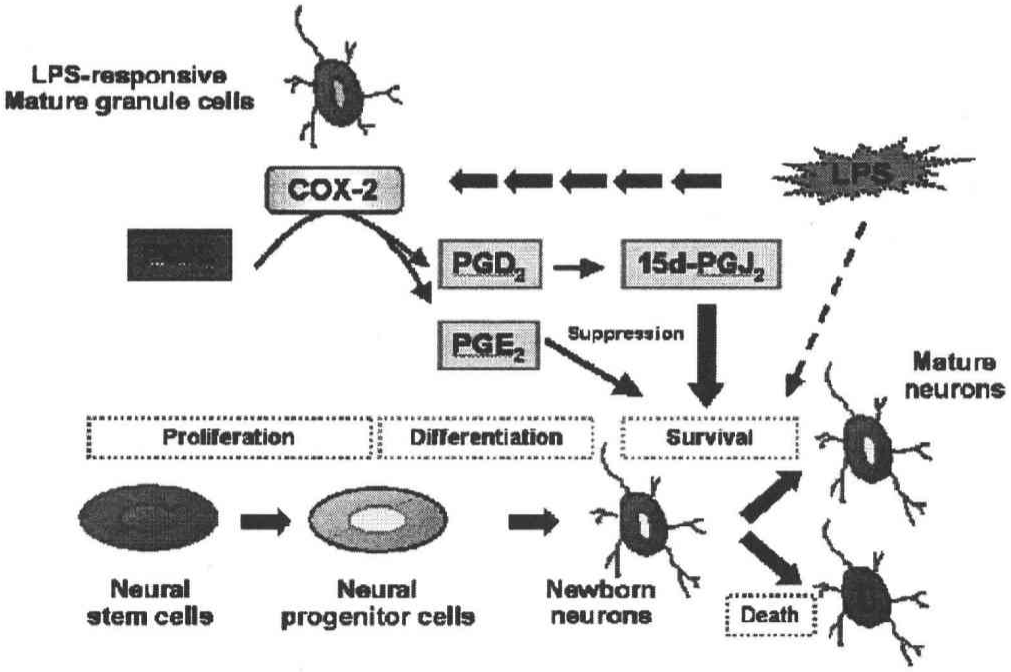

Fig. 1: The speculated diagram showing that LPS suppresses the neurogenesis in the adult mouse DG by impairing the newborn cell survival presumably via COX-2-mediated PGD_2 and 15d-PGJ_2 production.

審 査 結 果 の 要 旨

神経幹細胞は自己複製能と神経細胞やグリア細胞への分化能を有する細胞であるが，発生期のみならず成体脳にも存在することが明らかになり，そのうちの海馬歯状回では，毎日数千の新生細胞が生まれていると考えられている。神経幹細胞の自己複製能や分化能，さらに新生した細胞の生存能などは，虚血，てんかん，神経炎症などで影響を受けることが知られており，これらの機能を調節することが出来れば，多くの中枢神経系の疾病を治療することが可能になる。一方，バクテリアの内毒素であるリポポリサッカライド（LPS）は神経炎症を引き起こして神経新生に影響を与えることが報告されている。すなわち，LPS は神経新生を抑制するが，その詳細なメカニズムについては不明な点が多い。そこで，本論文では成体マウスの歯状回における神経新生が LPS 処理によってどのように影響されるかを検討し，そのメカニズムについても解析を加えた。

LPS をマウスに投与し，その 5 時間後に増殖マーカーのブロモデオキシウリジン（BrdU）を投与した。その 2 時間後に脳をサンプリングし，BrdU の取り込みを調べたが，影響は認められなかった。一方，LPS 投与後 7 日目あるいは 21 日目の BrdU 取り込みにより，神経幹細胞の分化あるいは生存を検討したところ，LPS 投与マウスの歯状回において BrdU 取り込みを示す細胞数の減少が見られた。また，LPS 処理により，TUNEL- 陽性細胞が増加していることも示された。すなわち，LPS は神経幹細胞から新生した細胞の生存を抑制していることが示された。一方，LPS は神経幹細胞からの神経細胞やアストロサイトへの分化には影響を与えなかった。したがって，LPS は神経幹細胞から生まれた未熟な新生細胞の生存を抑制するものと考えられた。次に，*in vitro* で海馬由来の神経幹細胞の生存に及ぼす LPS の作用について検討を加えたが，LPS は抑制を示さなかった。したがって，LPS の作用は神経幹細胞に直接影響を与えているのではなく，他の細胞を介した間接的な作用と考えられた。LPS の作用は NFκB を介してシクロオキシゲナーゼ -2（COX-2）を発現させ，プロスタノイドの生合成を高めることが知られている。そこで，LPS による新生細胞の生存抑制におよぼす COX-2 の介在について検討をしたところ，非特異的 COX 阻害薬のインドメタシンや COX-2 特異的阻害薬の NS-398 によって生存抑制の回復が見られ，COX-2 の介在が考えられた。次に，歯状回で COX-2 を発現している細胞種を検討したところ，ミクログリアではなく，成熟した神経細胞の一部であった。さらに，COX-2 によって生成するどの種のプロスタノイドが細胞の生存抑制に関与するのかを，*in vitro* の神経幹細胞を用いて検討したところ，プロスタグランジン D_2，その非酵素的代謝物の 15- デオキシ - プロスタグランジン J_2 およびプロスタグランジン E_2 に抑制活性が認められた。すなわち，これらのプロスタノイドを介して LPS が神経幹細胞からの新生細胞の生存を抑制していることが考えられた。以上のように，本研究は神経炎症を引き起こす LPS の神経幹細胞機能に及ぼす作用の一端を明らかにした極めて貴重なものである。

したがって，本論文は博士（薬学）の学位論文として合格と認める。