Neurotoxic Effects of Lipopolysaccharide on Nigral Dopaminergic Neurons Are Mediated by Microglial Activation, Interleukin-1β, and Expression of Caspase-11 in Mice*

Received for publication, June 30, 2004, and in revised form, September 21, 2004
Published, JBC Papers in Press, September 21, 2004, DOI 10.1074/jbc.M407328200

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The endotoxin lipopolysaccharide (LPS), a component of the Gram-negative bacterial cell wall, prospectively induces degeneration of substantia nigral (SN) dopaminergic neurons via activation of microglial cells in rats and mice. Caspase-11 plays a crucial role in LPS-induced septic shock in mice. We examined the mechanism of LPS neurotoxicity on SN dopaminergic neurons in C57BL/6 mice and caspase-11 knockout mice. Mice were stereotactically injected with LPS into the SN on one side and vehicle into the SN of the other side. Immunohistochemistry, Western blotting analysis, enzyme-linked immunosorbent assay, and reverse transcriptase-PCR were performed to evaluate damage of SN dopaminergic neurons and activation of microglial cells. Intranigral injection of LPS at 1 or 3 μg/μl/site decreased tyrosine hydroxylase-positive neurons and increased microglial cells in the SN compared with the contralateral side injected with vehicle at days 7 and 14 post-injection in C57BL/6 mice. Intranigral injection of LPS at 3 μg/μl/site induced the expression of caspase-11 mRNA in the ventral midbrain at 6, 8, and 12 h post-injection, and the expression of caspase-11-positive cells in the SN at 8 and 12 h post-injection. Moreover, LPS at 3 μg/μl/site increased interleukin-1β content in the ventral midbrain at 12 and 24 h post-injection. LPS failed to elicit these responses in caspase-11 knockout mice. Our results indicate that the neurotoxic effects of LPS on nigral dopaminergic neurons are mediated by microglial activation, interleukin-1β, and caspase-11 expression in mice.

Parkinson’s disease (PD)† is histologically characterized by degeneration of dopaminergic neurons in the substantia nigra (SN). High concentrations of microglial cells are present in the SN, and activation of these cells has been observed in the SN of PD patients (1, 2). Recent studies postulated that activation of microglia and neuroinflammatory processes may contribute to the pathogenesis of PD (3, 4). Lipopolysaccharide (LPS), a component of the Gram-negative bacterial cell wall, is a potent inducer of inflammation and activator of microglia (5). Long-term stimulation of innate immunity by LPS exacerbates motor neurodegeneration in a mouse model of amyotrophic lateral sclerosis, suggesting that the endotoxin LPS could be involved in neurodegenerative diseases caused by chronic peripheral bacterial infections especially in the presence of genetic or environmental risk factors (6).

Dopaminergic neurons are far more sensitive to LPS-induced neurotoxicity than γ-aminobutyric acidergic or serotonergic neurons, and intranigral injection of LPS induces selective and long-lasting dopaminergic neurodegeneration via microglial activation in the SN (5). In PD patients, microglial activation, and selective and irreversible dopaminergic neurodegeneration in the SN are seen (1, 2); therefore intranigral injection of LPS is a suitable model for PD (5). With regard to the signal transduction by LPS, caspase-11 is considered to play a major role because resistance to LPS-induced septic shock has been reported in caspase-11-deficient mice (7). However, the mechanism of LPS neurotoxicity of dopaminergic neurons in SN is not fully elucidated.

We previously demonstrated that overexpression of the Apaf-1 dominant-negative inhibitor suppressed the mitochondrial apoptotic cascade in chronic methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment (8), and knockout of caspase-11 protected against acute MPTP-induced dopaminergic neuronal death and activation of microglial cells through the inflammatory cascade (9). The present study was designed to elucidate the mechanism of SN dopaminergic neurodegeneration in a mouse model of hemi-Parkinson’s disease induced by intranigral injection of LPS. In particular, we focused on the role of caspase-11 in LPS-induced SN dopaminergic neurotoxicity in C57BL/6 mice and caspase-11 knockout mice.

MATERIALS AND METHODS

Animals—Male C57BL/6 mice (10–11 weeks old) were purchased from Charles River Laboratories (Kanagawa, Japan) and acclimated to the laboratory environment for 1 week before the experiment. Caspase-11 knockout mice were generated by gene-targeting techniques and back-crossing for at least five generations (usually eight generations) into the C57/Black background. Caspase-11 knockout mice were a generous gift from Prof. J. Yuan (Harvard Medical School, Boston, MA), and establishment of caspase-11 knockout mice was described in detail by Wang et al. (10). Homozygous absence of the caspase-11 gene in experimental animals was repeatedly checked by PCR using specific primers. The mice were kept on a 12/12 h light/dark advertisement
cycle with ad libitum access to food and water. All of the experiments were conducted using male mice (10–14 weeks old; weight, 25–30 g). All of the surgical procedures were performed according to the guidelines of the Ethics Committee for the Use of Laboratory Animals at Juntendo University.

Reagents—Lipopolysaccharide (serotype 026:B6, Escherichia coli) was purchased from Sigma. The rabbit polyclonal anti-tyrosine hydroxylase (TH) antibody was purchased from Calbiochem-Novabiochem Corp. (San Diego, CA). The rabbit polyclonal antibody against Iba1, a microglia/macrophage-specific calcium-binding protein (11), was kindly provided by Dr. Shinichi Kohsaka from the National Institute of Neuroscience (Tokyo, Japan). The rat monoclonal anti-caspase-11 antibody was a gift from Prof. J. Yuan (10). Biotinylated goat anti-rabbit and anti-rat secondary antibodies were purchased from Dako (Glostrup, Denmark) and Vector Laboratories Inc. (Burlingame, CA), respectively. VECTASTAIN ABC reagents were purchased from Vector Laboratories Inc. Monastral blue was purchased from Aldrich, and 3,3′-diaminobenzidine was purchased from Sigma.

LPS Injection—The mice were anesthetized with 70 mg/kg sodium pentobarbital and positioned in a stereotaxic apparatus (Narishige Scientific Instruments, Tokyo, Japan). Using the brain atlas of Paxinos and Franklin (12), LPS was injected into the SN using a 2-μl Hamilton microsyringe (Switzerland) into the following stereotaxic coordinates: 2.8 mm posterior to bregma, 1.3 mm lateral to the midline, and 4.5 mm ventral to the surface of the dura mater. LPS was dissolved in a solution of 1% Monastral blue in phosphate-buffered saline (PBS, pH 7.4) for the experiments of immunohistochemistry or PBS (pH 7.4) for the experiments of Western blotting analysis, enzyme-linked immunosorbent assay (ELISA), and reverse transcriptase (RT)-PCR and injected slowly into one side of the SN in a volume of 1 μl over a period of 1 min, and the needle was kept in place for 3 min after injection. Vehicle (1% Monastral blue in PBS or PBS) was injected in a similar manner into the contralateral SN. Monastral blue was used as an inert tracer of injection site according to the method of Herrera et al. (5).

Immunohistochemistry—The mice were perfused transcardially with PBS followed by ice-cold 4% paraformaldehyde in PBS (pH 7.4). The brains were removed and then immersed in 4% paraformaldehyde for 2 days at 4 °C and then in 30% sucrose containing 0.01% sodium azide for 24 h at 4 °C. Coronal sections (20-μm thick) were cut using a microtome and stored in 30% sucrose containing 0.01% sodium azide. In all immunostaining procedures, the sections were first rinsed with PBS containing 0.05% Tween 20 (PBS-T). They were then immersed in a solution of 3% H2O2, 10% methanol in PBS-T for 10 min. After washing (three times with PBS-T), the sections were treated with blocking solution (PBS-T containing 10% Block-Ace; Yukiirushi Co., Sapporo, Japan) for 30 min. The sections were incubated overnight at 4 °C with the primary antibodies diluted with blocking solution (anti-TH, 1:2,000; anti-Iba1, 1:10,000; anti-caspase-11, 1:100). The sections were again washed with PBS-T (3 times) and incubated with the appropriate biotinylated secondary antibodies (1:500) in blocking solution for 1 h at room temperature. The sections were washed three times with PBS-T in between the steps. The bound complex was visualized with 3,3′-diaminobenzidine and urea-hydrogen peroxide tablets from Sigma dissolved in water. Color development was terminated by removing the reagents and washing the sections with PBS (twice). As a negative control, immunohistochemistry was performed without the primary antibodies. Regarding immunohistochemistry for caspase-11, the nigral sections from caspase-11 knockout mice

![Diagram](image-url)

**Fig. 1.** Intranigral injection of LPS decreased the number of TH-positive cells in the SN at day 7 post-injection in C57BL/6 mice. A, side injected with 1 μl of 1% Monastral blue in PBS. B, side injected with LPS (1 μg in 1 μl of 1% Monastral blue in PBS). C and D, large magnifications of squares in A and B, respectively. Dye indicates the injection site. Scale bars, 200 μm for A and B; 100 μm for C and D. E, effects of LPS on the number of TH-positive cells in the SN at days 7 and 14 post-injection of LPS 1 or 3 μg in 1 μl of 1% Monastral blue in PBS in C57BL/6 mice. Each value represents the mean ± S.E. *, p < 0.05; **, p < 0.01 versus the control side (Student’s t test, n = 3–6 mice).
were also stained with anti-caspase-11 antibody as a negative control. The images were analyzed with an Olympus CX40 microscope (Tokyo, Japan) connected to a digital camera (DXM1200, Nikon, Tokyo, Japan) and analyzed with the ACT-1 image system (version 2.20, Nikon, Tokyo, Japan).

Cell Counting—To examine the effects of LPS on the density of dopaminergic neurons and activation of microglial cells, nigral sections were prepared from the identifiable level of the third cranial nerve rootlets, which is a good index for degeneration of nigral dopaminergic neurons (13). The number of TH-positive neurons in the LPS-injected side was compared with the vehicle-injected side at the level of third cranial nerve rootlets in substantia nigra.

Western Blotting Analysis—The mice were perfused transcardially with PBS, and ventral midbrain tissues were removed. Total protein extracts of the ventral midbrain were obtained by homogenization in CellLytic-MT Mammalian Tissue Lysis/Extraction reagent (Sigma) with a mixture of protease inhibitor mixture set (Calbiochem, La Jolla, CA). The protein concentration in the tissue lysates was determined using a BCA protein assay kit (Pierce). An equal amount of protein for each sample was separated by SDS-PAGE (10 or 15% gels; Bio-Rad) and then transferred onto polyvinylidene fluoride membranes (Bio-Rad). The membranes were blocked with 5% Block-Ace in PBS and sequentially incubated with antibodies against TH (Calbiochem, 1:2,000), Iba1 (gift from Dr. Kohsaka, 1:10,000), caspase-11 (gift from Prof. Yuan, 1:100), and actin (mouse monoclonal; Santa Cruz Biotechnology; 1:100) and then with horseradish peroxidase-conjugated anti-rabbit, anti-rat, or anti-mouse antibody (Amersham Biosciences). Western blots were identified by ECL plus Western blotting Detection System (Amersham Biosciences). Images were analyzed with Intelligent Dark Box II (LAS-1000; Fuji Film, Tokyo, Japan).

Determination of Maturity IL-1β Content in the Ventral Midbrain—The mice were perfused transcardially with PBS, and ventral midbrain tissues were carefully removed. The tissues were weighed, and the total protein extracts of the ventral midbrain were obtained by homogenization in CellLytic-MT mammalian tissue lysis/extraction reagent (Sigma) with a protease inhibitor mixture set (Calbiochem). The amount of matured IL-1β was quantified using an ELISA kit specific for the mature form of the cytokine (R & D Systems, Minneapolis, MN). The content of mature IL-1β in the ventral midbrain was expressed as the amount/mg of tissue.

Total RNA Extraction and RT-PCR—The mice were perfused transcardially with PBS, and ventral midbrain tissues were carefully removed. Total RNAs from the ventral midbrain of C57BL/6 mice were prepared using an RNeasy kit (Qiagen). The concentration and purity of the RNA preparations were determined by measuring the absorbance at 260 and 280 nm by spectrophotometer. First strand cDNA was synthesized from total RNA using a first strand cDNA synthesis kit (SuperScript, Invitrogen, CA) according to the instructions provided by the manufacturer. The cDNA template was then amplified by PCR using AmpliTaq Gold (Applied Biosystems). The nucleotide sequences of the primers were based on published cDNA sequences of caspase-11 (forward, 5'-AGAAGTCTTACGGAGTACC-3'; reverse, 5'-TGTTGTTCTTGAGAGTCCGCAGC-3'). It is reported that genomic DNA of caspase-11 includes at least 8 exons (10), and our primers were designed between exons 2 and 5 (525 bp). As an internal control, β-actin cDNA was co-amplified using the primers 5'-ACCTGAGACCTCTCATGC-3' (forward) and 5'-AACCGACGTCATGAACAGTG-3' (reverse). After amplification, the products were separated on 1% agarose gel containing ethidium bromide. The bands were then visualized under UV transillumination.

Statistical Analysis—The data are expressed as the means ± S.E. The differences between groups were examined for statistical significance using Student's t test. A p value less than 0.05 denoted the presence of a statistically significant difference.

RESULTS
LPS Neurotoxicity to the SN Dopaminergic Neurons in C57BL/6 Mice—In immunohistochemical studies, LPS (1 or 3 μg in 1 μl of 1% Monastral Blue in PBS) was stereotaxically injected into the SN to examine its effect on neurodegeneration. Examination at day 7 after intranigral injection of LPS at 1 μg/μl/site showed a decrease in the number of TH-positive cells compared with the count in the contralateral SN injected with 1% Monastral Blue in PBS (Fig. 1, A and B). In addition to the loss of cell bodies, the fibers of TH-positive cells in the SN were also damaged (Fig. 1, C and D). However, LPS injection did not significantly alter the number and integrity of the TH-positive cells in the ventral tegmental area, an area adjacent to the SN (Fig. 1, A and B). The dye indicates the site of injection (Fig. 1, A–D). Quantitative analysis showed that injection of LPS at 1 and 3 μg/μl/site reduced the number of TH-positive cells in the SN at 7 days and 14 post-injection (Fig. 1E).

In Western blotting analysis, LPS (1 and 3 μg in 1 μl of PBS) was stereotaxically injected into the SN to examine its effect on
TH level in the ventral midbrain. Examination at day 7 after intranigral injection of LPS at 1 and 3 μg/μl/site showed a decrease in the TH level in the ventral midbrain compared with the level in the contralateral region injected with PBS (Fig. 2).

**LPS Induces SN Microglial Activation in C57BL/6 Mice**—In immunohistochemical studies, LPS (1 μg in 1 μl of 1% Monastral Blue in PBS) was stereotaxically injected into the SN to examine its effect on microglial activation using anti-Iba1 (a microglia/macrophage-specific calcium-binding protein) antibody. Activation of microglia was observed in the LPS-injected side compared with the vehicle-injected side at day 7 post-injection (Fig. 3, A–D).

In Western blotting analysis, LPS (3 μg in 1 μl of PBS) was stereotaxically injected into the SN to examine its effect on Iba1 level in the ventral midbrain. Intranigral injection of LPS at 3 μg/μl/site resulted in an increase in Iba1 level in the ventral midbrain at days 4 and 7 post-injection and a decrease in TH level in the same area at day 7 post-injection. (Fig. 3E).

**LPS Increases IL-1β Content in the Ventral Midbrain of C57BL/6 Mice**—LPS (3 μg in 1 μl of PBS) was stereotaxically injected into the SN to examine its effect on mature IL-1β level in the ventral midbrain by ELISA. At 12 and 24 h post-injection, LPS (3 μg/μl/site) dramatically increased mature IL-1β content in the ventral midbrain compared with the contralateral side injected with PBS (Fig. 4).

**LPS Induces Caspase-11 mRNA Expression in the Ventral Midbrain of C57BL/6 Mice**—It is reported that caspase-11 contributes to the secretion of IL-1β (10). We examined the caspase-11 mRNA expression by RT-PCR to investigate the hypothesis that LPS causes increased caspase-11 expression and thereby facilitates IL-1β production. LPS (3 μg in 1 μl of PBS) was stereotaxically injected into the SN. At this dose, LPS induced the expression of caspase-11 mRNA in the ventral midbrain at 6, 8, and 12 h post-injection in C57BL/6 mice (Fig. 5A). At 4 h post-injection of LPS (3 μg in 1 μl of PBS), the level of caspase-11 mRNA in the ventral midbrain was very low and similar to the level of the midbrain of untreated control mice (Fig. 5A).

**LPS Induces Caspase-11 Expression in the SN or Ventral Midbrain of C57BL/6 Mice**—We examined the caspase-11 expression by immunohistochemistry and Western blotting analysis to investigate the hypothesis that LPS causes increased caspase-11 expression and thereby facilitates IL-1β production. In immunohistochemical studies, we stereotaxically injected LPS (3 μg in 1 μl of 1% Monastral Blue in PBS) into the SN to examine the expression of caspase-11 in the SN. At this dose, LPS induced the expression of caspase-11 in the SN at 8 and 12 h post-injection in C57BL/6 mice (Fig. 5, B and C). Caspase-11 was observed in microglia and/or neuron-like cells at 8 and 12 h post-injection in the SN. The positive cells were completely absent in the SN of caspase-11 knockout mice (Fig. 5D). Moreover, positive cells were also completely absent at 12 h post-injection in the SN of C57BL/6 mice in case of immunostaining without anti-caspase-11 antibody (data not shown).

In Western blotting analysis, we stereotaxically injected LPS (3 μg in 1 μl of PBS) into the SN to examine the expression of caspase-11 in the ventral midbrain. At this dose, LPS induced the expression of caspase-11 in the ventral midbrain at 12 h post-injection but not 4 h post-injection in C57BL/6 mice (Fig. 5E). The anti-caspase-11 monoclonal antibody used in the present study recognizes two proteins of 43 and 38 kDa in LPS-stimulated tissue (10, 14). In the present study, we could see two proteins induced by LPS at 12 h post-injection in C57BL/6 mice (Fig. 5E).

**Caspase-11 Knockout Mice Are Resistant to LPS-induced Degeneration of TH-positive Cells**—To examine the effect of LPS on neurodegeneration, we stereotaxically injected LPS (3 μg in 1 μl of 1% Monastral Blue in PBS) into the SN of caspase-11 knockout mice and wild type mice. Seven days after intranigral injection of LPS, the loss of TH-positive cells was not observed in caspase-11 knockout mice compared with same region in wild type mice (Fig. 6, A–F). Caspase-11 knockout mice were resistant to any decrease in TH-positive cells compared with the wild type mice. The number of TH-positive cells in the SN did not change after injection of LPS in caspase-11 knockout mice (Fig. 6G). For Western blotting analysis, LPS (3 μg in 1 μl of PBS) was stereotaxically injected into the SN to examine its effect on TH level in the ventral midbrain of caspase-11 knockout mice and wild type mice. Examination at day 7 after intranigral injection showed that LPS failed to reduce the level of TH in the ventral midbrain compared with contralateral region (Fig. 6H).

**Caspase-11 Knockout Mice Are Resistant to LPS-induced Activation of Microglia**—We stereotaxically injected LPS (3 μg in 1 μl of 1% Monastral Blue in PBS) into the SN of caspase-11 knockout mice and wild type mice to examine the effect of LPS on microglial activation in the SN. LPS induced only a moderate microglial activation in the SN compared with same region of the wild type mice at day 7 post-injection (Fig. 7, A and B).

**Caspase-11 Knockout Mice Are Resistant to LPS-induced Elevation of Mature IL-1β Content in the Ventral Midbrain**—To examine the effects of LPS on mature IL-1β level in the ventral midbrain of caspase-11 knockout mice by ELISA, LPS was stereotaxically injected at 3 μg in 1 μl of PBS into the SN. Such injection failed to increase IL-1β content; its level was below detection at 12 and 24 h post-injection (Table I).
DISCUSSION

In the present study, we confirmed that intranigral injection of LPS induced the expression of caspase-11 mRNA in the ventral midbrain at 6, 8, and 12 h post-injection by RT-PCR and the expression of caspase-11 in the SN at 8 and 12 h post-injection by immunohistochemistry and/or Western blotting analysis. We also demonstrated that LPS decreased the number of TH-positive cells in the SN via microglial activation and IL-1β release in C57BL/6 mice. Moreover, caspase-11 knockout mice were resistant to these LPS-induced responses. Our results suggest that caspase-11 mediates, at least in part, the LPS-induced neurotoxicity on SN dopaminergic neurons in mice.

It is well known that LPS, a major component of the outer membrane of Gram-negative bacteria, is an endotoxin that induces inflammatory response through cytokine production (10). Recent studies reported that the innate immune system in the brain could be activated during endotoxemia; intraperitoneal injection of LPS stimulated the expression of genes encoding IL-6 and its receptor in selective regions of the brain (15, 16). It is also reported that intraperitoneal injection of LPS caused a marked increase in the expression of Toll-like receptor 2 mRNA across the brain and spinal cord (6, 17). Moreover, Nguyen et al. (6) demonstrated that repeated systemic injections of LPS exacerbated motor neurodegeneration in a mouse model of amyotrophic lateral sclerosis.

The SN is a more sensitive area to LPS than the cortex and hippocampus, both in vivo and in vitro, probably because of the high proportion of microglia in the SN (18). Moreover, other studies indicated that intranigral injection of LPS selectively induced the death of dopaminergic neurons via activation of microglial cells in the SN but not of γ-aminobutyric acidergic and serotonergic neurons (5, 19). In addition, the damage to dopaminergic SN neurons by intranigral injection of LPS was long lasting; it was still observed one year post-injection (5). These observations by intranigral injection of LPS are similar to the characteristic features of PD because microglial activation, and selective and irreversible dopaminergic neurodegeneration in the SN are also seen in PD patients (1, 2). Taking these results together, intranigral injection of LPS is a suitable model for PD on the viewpoint of similarity, that is selective and long lasting dopaminergic neurodegeneration via microglial activation in the SN without systemic effects (5, 13, 18–22).

In the present study, we used the nigral sections at the level of the third nerve rootlets for evaluation of TH-positive cells in the SN on the LPS-injected side and the vehicle-injected side. In this context, Iravani et al. (13) suggested previously that cell counting using nigral sections at the easily identifiable level of the third nerve rootlets was a good index for evaluating changes in TH-positive cells in the SN.

In the present study, LPS induced the expression of caspase-11 in the SN and such expression preceded IL-1β secretion in the ventral midbrain, suggesting the involvement of caspase-11 in IL-1β secretion. Murine caspase-11 is a member of the caspase-1 subfamily (14). Caspase-1 is involved in processing pro-IL-1β to mature IL-1β, and the activation of FIG. 5. A, effects of LPS on caspase-11 mRNA expression in the ventral midbrain by RT-PCR. Intranigral injection of LPS (3 μg in 1 μl of PBS) induced caspase-11 mRNA expression at 6, 8, and 12 h post-injection in the ventral midbrain of C57BL/6 mice. At 4 h post-injection of LPS, the level of caspase-11 mRNA in the ventral midbrain was low and same as that of untreated mouse. Cont, control: ventral midbrain from untreated mouse. B and C, effects of LPS on caspase-11 expression in the SN by immunohistochemistry. Intranigral injection of LPS (3 μg in 1 μl of 1% Monastral Blue in PBS) induced caspase-11 expression at 8 (B) and 12 h (C) post-injection in the SN of C57BL/6 mice. D, LPS failed to induce caspase-11 expression at 12 h post-injection in the SN of caspase-11 knockout mice. Immunohistochemistry was performed by the ABC method. Microglia-like cells (arrow) and a neuron-like cell (arrowhead) were observed (n = 2). KO, knockout. Scale bar, 20 μm for A–C. E, Western blotting analysis of the effects of LPS on caspase-11 expression in the ventral midbrain. Intranigral injection of LPS (3 μg in PBS) induced caspase-11 expression at 12 h post-injection in the ventral midbrain of C57BL/6 mice but not 4 h post-injection. The data are representative of three experiments.

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caspase-1 is induced by caspase-11; therefore caspase-11 is essential for IL-1β secretion (10). Moreover, caspase-11 plays a major role in LPS-induced inflammatory response; caspase-11 knockout mice are resistant to LPS-induced septic shock (7, 10). Previous studies demonstrated the expression of caspase-11 in brain neurons and microglia (9, 23); caspase-11 appears to play a crucial role in MPTP mouse models of PD (9). In the present study, expression of caspase-11 was observed in both microglial cells and neurons. It is suggested that caspase-11 in microglial cells and neurons contribute to IL-1β secretion (23) and apoptosis (24), respectively. Therefore both inflammatory process and apoptotic pathway seem to participate in LPS-induced SN dopaminergic neurotoxicity. These findings, together with our results, suggest that caspase-11 is involved in selective dopaminergic neurodegeneration in the SN in both MPTP and LPS models.

In patients with PD, high levels of IL-1β were detected in the striatum (25) and cerebrospinal fluid (26). MPTP treatment resulted in overexpression of IL-1β mRNA, and caspase-11 knockout mice were resistant to this effect (9), suggesting that IL-1β is a key factor in MPTP neurotoxicity for SN dopaminergic neurons. The dominant-negative caspase-1 also inhibited SN dopaminergic neuronal death induced by MPTP (27). These findings indicate that IL-1β is a key cytokine in the MPTP model of Parkinson’s disease. In the present study, LPS caused marked increase in IL-1β content in the ventral midbrain of C57BL/6 mice, and caspase-11 knockout mice did not show any increase in IL-1β secretion in response to LPS, suggesting the involvement of IL-1β in LPS neurotoxicity of the SN, similar to its role in MPTP model. In Fig. 4, a small increase in mature IL-1β content was observed in the contralateral side injected with PBS. It is supposed that microglial cells can migrate and move in the brain (28). Therefore the small increase in the IL-1β level in the contralateral region may be contributed by the microglial cells that moved to contralateral side by stimulation of LPS because PBS injection alone did not affect the mature IL-1β level (Fig. 4).

Activation of microglia is a major event in LPS-induced SN dopaminergic neurodegeneration in rats and mice (5, 18–22, 29). In the present study, we also observed LPS-induced activation of microglia, suggesting that these cells are important in LPS-induced SN neurodegeneration. Microglia and macrophages are located in a close position in the differentiation pathway (30), but whether peripheral macrophages convert to microglia after injection of LPS remains to be determined. In this regard, Lehnardt et al. (31) suggested that microglial cells expressed Toll-like receptor 4 in the CNS, and Toll-like receptor 4-mediated LPS-induced neuronal damage and cell death.
the pathogenesis of PD. Hence, the LPS endotoxin is a useful tool for examination of the inflammatory process in PD based on activation of microglia by bacterial infection.

In conclusion, we demonstrated in the present study that intranigral injection of LPS induced the expression of caspase-11, decreased the number of TH-positive cells, increased microglial cell counts in the SN, and increased IL-1β content in the ventral midbrain in C57BL/6 mice. Moreover, caspase-11 knockout mice were resistant to the effects of LPS. These results suggest that caspase-11 plays a major role in LPS-induced substantia nigral dopaminergic neurodegeneration in mice.

Acknowledgments—We are grateful to Prof. Junying Yuan (Harvard Medical School, Boston, MA) for providing caspase-11 knockout mice and anti-caspase-11 antibody and to Dr. Shinichi Kohsaka (National Institute of Neuroscience, Tokyo, Japan) for providing anti-Iba1 antibody. We thank Dr. Kenji Yoshimi for helpful advice and suggestions on the present study.

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Table I

| Time relative to LPS injection | IL-1β content in ventral midbrain | Wild type | Caspase-11 knockout |
|-----------------------------|-----------------------------|----------|---------------------|
| Before                      | Under detectable level      | ND       | Under detectable level |
| 12 h after                  | 29.9 ± 3.3*                 | Under detectable level |
| 24 h after                  | 38.6 ± 2.2*                 | Under detectable level |

* ND, not determined.

b p < 0.01 versus respective vehicle side by Student’s t test (n = 2–6).

α p < 0.001 versus respective vehicle side by Student’s t test (n = 2–6).

A. Wild type

B. Caspase-11 knockout

FIG. 7. LPS (3 μg in 1 μl of 1% Monastral Blue in PBS) fails to activate microglia in the SN of caspase-11 knockout mice at day 7 post-injection. A, wild type mice. B, caspase-11 knockout mice. Iba1, a microglia/macrophage-specific calcium-binding protein, was used as a marker for microglia. Vehicle, 1% Monastral blue in PBS. Immunohistochemistry was performed by the ABC method (n = 3 mice). Scale bar, 100 μm for A and B.