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THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 278, No. 33, Issue of August 15, pp. 30732–30740, 2003
Printed in U.S.A.

Maturation and Release of Interleukin-1β by Lipopolysaccharide-primed Mouse Schwann Cells Require the Stimulation of $P2X_7$ Receptors*

Received for publication, April 30, 2003, and in revised form, June 4, 2003
Published, JBC Papers in Press, June 9, 2003, DOI 10.1074/jbc.M304534200

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The $P2X_7$ receptor, mainly expressed by immune cells, is a ionotropic receptor activated by high concentration of extracellular ATP. It is involved in several processes relevant to immunomodulation and inflammation. Among these processes, the production of extracellular interleukin-1β (IL-1β), a pro-inflammatory cytokine, plays a major role in the activation of the cytokine network. We have investigated the role of $P2X_7$ receptor and of an associated calcium-activated potassium conductance (BK channels) in IL-1β maturation and releasing processes by Schwann cells. Lipopolysaccharide-primed Schwann cells synthesized large amounts of pro-IL-1β but did not release detectable amounts of pro or mature IL-1β. ATP on its own had no effect on the synthesis of pro-IL-1β, but a co-treatment with lipopolysaccharide and ATP led to the maturation and the release of IL-1β by Schwann cells. Both mechanisms were blocked by oxidized ATP. IL-1β-converting enzyme (ICE), the caspase responsible for the maturation of pro-IL-1β in IL-1β, was activated by $P2X_7$ receptor stimulation. The specific inhibition of ICE by the caspase inhibitor Ac-Tyr-Val-Ala-Asp-aldehyde blocked the maturation of IL-1β. In searching for a link between the $P2X_7$ receptor and the activation of ICE, we found that enhancing potassium efflux from Schwann cells upregulated the production of IL-1β, while strongly reducing potassium efflux led to opposite effects. Blocking BK channels actually modulated IL-1β release. Taken together, these results show that $P2X_7$ receptor stimulation and associated BK channels, through the activation of ICE, leads to the maturation and the release of IL-1β by immune-challenged Schwann cells.

Interleukin-1 is a pro-inflammatory cytokine that mediates part of the host defense response to injury and infection. It is produced by activated monocytes and macrophages (1), but also by microglia in the central nervous system (2). Both forms of IL-1,[1] *i.e.* IL-1α and IL-1β are synthesized as precursor molecules of 31–35 kDa, which are cleaved by proteolytic enzymes into a mature form of about 17 kDa. While IL-1α is biologically active in both forms (pro-IL-1α and mature IL-1α), IL-1β is only active when converted in its mature form. The conversion of pro-IL-1β into mature IL-1β is achieved by a cysteine protease belonging to the caspase family, the IL-1β-converting enzyme (ICE) (3, 4), also termed caspase-1 (5). One striking feature of IL-1β is its lack of signal sequence, which normally targets proteins to the endoplasmic reticulum to be exported in the extracellular compartment (6, 7).

Despite numerous studies, the mechanisms of IL-β post-translational processing, are still ill defined. Because of the lack of signal peptide, it was first proposed that apoptosis of IL-1β-producing cells could be responsible for the release of IL-β (8), and this theory is still largely accepted (for review, see Ref. 9). However, macrophages and monocytes can release IL-1β without obvious signs of cell death (10, 11). This non-cytolytic production of IL-1β led to the proposal of other mechanisms like the exportation by specific transporters (12), the release from endosomal vesicles (13) or by microvesicle shedding (11). In most cases, the sole antigenic stimulation is poorly effective to release IL-1β. Indeed in generic macrophages or monocytes, pro-IL-1β tends to accumulate into the cytosol instead of being secreted, whereas mature IL-1β is absent from the intracellular compartment. It seems therefore that maturation and release processes are structurally and/or timely linked, possibly through the association of ICE subunits with the plasma membrane (14). Perregaux and Gabel (15) were first to demonstrate that nigericin, a potassium ionophore, or ATP potentiated greatly the release of IL-1β from LPS-primed macrophages. Since this pioneer work, *in vitro* but also *in vivo* studies (16, 17) have shown that extracellular ATP acting on the $P2X_7$ receptor, an ionotropic receptor that plays a pivotal role in the modulation of immune and inflammatory responses (9), was a very potent agent to stimulate the production of large quantities of extracellular mature IL-1β.

To unravel the mechanisms occurring downstream the $P2X_7$ receptor activation and triggering the production and the release of IL-1β, we have chosen to study mouse Schwann cells for two main reasons: 1) their importance as partially immune-competent cells within the peripheral nervous system and 2) the peculiarity of their $P2X_7$ receptors. Indeed, in addition to their well documented roles in myelination, trophic, and metabolic support of the neuronal network, Schwann cells are able to present antigens to immunocompetent cells by expressing

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[1] The abbreviations used are: IL, interleukin; ICE, IL-1β-converting enzyme; oATP, oxidized ATP; YVAD-CHO, Ac-Tyr-Val-Ala-Asp-aldehyde; FITC-VAD-FMK, fluoroisothiocyanate-Val-Ala-Asp-*O*-methlyfluoromethylketone; LPS, lipopolysaccharide; TLR4, toll-like receptor 4; LDH, lactate dehydrogenase; ELISA, enzyme-linked immunosorbent assay; AEBSF, 4-(2-aminoethyl)benzenesulfonylfluoride hydrochloride; RT, reverse transcriptase; $\beta_2$mgl, β_2-microglobulin; ChTx, charybdotoxin; TEA, tetraethylammonium.

major histocompatibility complex class II molecules under inflammatory conditions both *in vitro* (18) and *in vivo* (19). They produce chemokines (macrophage chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1α (MIP-1α); Ref. 20) and cytokines (both pro- and anti-inflammatory, for review see Ref. 21; Ref. 22). *In vitro*, Schwann cells synthesize IL-1β but poorly release it when challenged by an immune stimulus such as LPS (23). *In vivo*, Schwann cells in the course of experimental autoimmune neuritis the murine model for the human Guillain-Barre syndrome (24) or Wallerian degeneration following an axonal injury (25) are producing IL-1β. A specific feature of P2X$_7$ receptors expressed by cultured mouse Schwann cells are there association with a calcium-activated potassium conductance and a chloride conductance (26). This intriguing phenotype makes the study of P2X$_7$ receptors expressed by Schwann cells particularly interesting as the different conductances activated by extracellular ATP could participate to an integrated regulation of inflammatory processes occuring in the peripheral nervous system.

The aim of the present work was to investigate the role of the P2X$_7$ receptor and the associated conductances in IL-1β post-translational processing in LPS-primed Schwann cells. We found that P2X$_7$ receptor activation, through the activation of ICE, was essential to the production and the release of IL-1β. Potassium fluxes, through the P2X$_7$ receptor itself and through calcium-activated potassium channels, were linking the P2X$_7$ receptor activation to IL-1β processing. These results provide new insights on IL-1β processing mechanisms and on how Schwann cells, the main glial cells of the peripheral nervous system, can sense an immune stimulation and respond to it by activating the cytokine network.

EXPERIMENTAL PROCEDURES

Glial Cell Cultures—Schwann cells were cultured from excised dorsal root ganglia from OF1 mouse embryos (E19) as described previously (27). Briefly, mice were killed by decapitation, and dorsal root ganglia were aseptically removed from embryos and plated onto 35-mm Petri dishes (Nunc, Strasbourg, France) coated with rat tail collagen type 1 (Upstate Biotechnology, Lake Placid, NY). Cells were cultured for the first 3 days in modified Eagle's medium (Invitrogen, Paisley, UK) containing nerve growth factor (100 ng/ml, Alomone, Jerusalem, Israël) and a mixture of uridine (10 μM) and fluorodeoxyuridine (10 μM) to suppress dividing fibroblasts. Then, cells were cultured with α-modified Eagle's medium (Invitrogen) containing nerve growth factor (20 ng/ml). The medium was changed twice a week, and cells were used between 4 and 6 weeks of culture. In some experimental conditions, ganglia were excised before measuring intracellular and extracellular IL-1β to eliminate an eventual neuronal source of IL-1β.

Primary glial cells from mouse brain were used as a positive control for the expression of Toll-like receptors 4 (TLR4). They were cultured from newborn mice as previously described in detail (28) and were plated at a density of 5×10^4 cells/dish into Dulbecco's modified Eagle's medium containing 20% heat-inactivated fetal calf serum (Roche Molecular Biochemicals; <10 pg ml^{-1} endotoxins). Under these conditions, neurons do not survive the mechanical dissociation, and the low plating density prevents oligodendrocyte proliferation.

Cell Treatments—Schwann cell cultures were primed for 6 h with LPS (10 μg/ml, *Escherichia coli*, serotype 0127B8, batch 63H4010, Sigma, St. Quentin Fallavier, France) with or without ATP (5 mM) during the last 30 min of stimulation. In some experiments, oxidized ATP (oATP) (300 μM), a P2X$_7$ receptor antagonist, was used during the last 90 min of stimulation. High external potassium solution (90 mM K^+) was obtained by adding 85 mM K^+ to the culture medium during the last 30 min, while K^+-free condition was obtained by replacing the culture medium by a nominal K^+-free α-modified Eagle's medium during the last 30 min of the protocol. In some experiments, a specific inhibitor of caspase 1 (Ac-Tyr-Val-Ala-Asp-CHO (YVAD-CHO)) (50–100 μM; Bachem, Voisins-les-Bretonneux, France) was added to block IL-1β maturation.

Detection of Intracellular and Extracellular IL-1β by ELISA—The concentrations of IL-1β (both pro and mature forms) released in the culture medium and present in cell lysates (*i.e.* intracellularly) were quantified by specific mouse IL-1β sandwich ELISAs. ELISA reagents were kindly supplied by Dr. S. Poole (National Institute for Biological Standard and Controls, Potters Bar, UK). Assay detection limits were <2 pg/ml. Following stimulation by LPS and ATP, media were collected, and a mixture of antiproteases was added to avoid protein degradation (4-(2-aminoethyl)benzenesulfonylfluoride hydrochloride (AEBSF), 0.2 mM; EDTA, 0.1 mM; bestatin, 13 μM; E64 0.14 μM; leupeptin, 0.1 μM; aprotinin, 0.03 μM). Extracellular protein contents were concentrated 20 times with Ultrafree-4 centrifugal filter units with a molecular mass cutoff of 4000 Da (Millipore, St. Quentin en Yvelines, France). Cell lysates were obtained by scratching cell cultures in a phosphate-buffered saline buffer containing 0.1% Triton X-100 and protease inhibitors. Cell debris and organelles were removed by centrifugation (12,000 × *g*, 10 min, 4 °C). Supernatants were assayed for intracellular IL-1β content. A sheep anti-mouse IL-1β antibody was used as a coating antibody, and a biotinylated sheep anti-mouse IL-1β antibody was used to sandwich the protein. Revelation was performed with horseradish peroxidase and *o*-phenylenediamine and read on a spectrophotometer. Both anti-IL1β antibodies recognize, without distinction, pro-IL-1β and mature IL-1β.

Immunoblot Analysis—Because IL-1β antibodies recognized both pro and mature forms of IL-1β, Western blot analysis were performed to differentiate them according to their molecular weight. After appropriate stimulation, media were collected using the same protocol than for ELISA assays and concentrated 100 times. Cell lysates were obtained by scratching cell cultures in a lysate buffer containing (in mM): Tris-HCl, 20; EDTA, 1; $MgCl_2$, 5; dithiothreitol, 1; aprotinin, 0.003; AEBSF, 1; sodium orthovanadate, 2 (pH 7.5) followed by mechanical trituration. Cell debris was removed by centrifugation (80 × *g*, 10 min, 4 °C), and supernatants were collected. Protein concentrations were determined by a colorimetric assay using bicinchoninate (MicroBCAssay, Interchim, Montluçon, France). 50 μg of protein were loaded into wells of a 13% acryl/bisacrylamide gel, and after separation, proteins were transferred to a polyvinylidene difluoride membrane (Immobilon-P, Millipore). After saturation in Tris-buffered saline-Tween (0.1%) containing 1% milk, the membrane was probed with a polyclonal sheep anti-mouse IL-1β (R&D Systems, Abingdon Oxon, UK) at 1:200 dilution and was incubated overnight at 4 °C and followed by 2-h incubation in a peroxidase-conjugated antibody-sheep IgG (1:8000). Revelation was obtained by chemoluminescence reaction (ECL, Amersham Biosciences, Orsay, France).

In Situ Detection of Caspase Activity—Caspase activity was revealed by FITC-VAD-FMK assay (Promega, Charbonnières, France). FITC-VAD-FMK is a fluorogenic substrate of caspases that binds only active caspases. FITC-VAD-FMK (10 μM) was added to treated cell cultures during 45 min, and cell cultures were fixed with PFA 4% during 10 min. Fluorescence was monitored using an excitation filter (wavelength: 400–440 nm) and a barrier filter (wavelength: 480 nm).

Electrophysiology—Currents were recorded from Schwann cells using the whole-cell configuration of the patch clamp technique. Patch pipettes were pulled from borosilicate glass capillaries (GF 150 TF-10, Clarck Electromedical Instruments, Pangbourne, UK) and filled with an internal solution containing (in mM): KCl, 120; $CaCl_2$, 1; $MgCl_2$, 2; HEPES, 10; EGTA, 10; glucose, 11; NaOH, 2; KOH, 33; (pH 7.4). In control conditions, the bathing solution (PSS) was (in mM): NaCl, 140; $CaCl_2$, 5; $MgCl_2$, 2; HEPES, 10; glucose, 11 and NaOH, 4 (pH 7.4). Voltage clamp protocols were applied from a holding potential of −70 mV by using a L/M-EPC-7 patch clamp amplifier (List Electronics, Darmstadt, Germany). Signals were stored on a digital audio tape recorder (DTR-1200, Biologic, Grenoble, France). Acute application of ATP was achieved to Schwann cells by a perfusion system based on electromagnetic valves controlling gravity flow (29).

Cell Viability—Cell death was assessed by colorimetric assay (Sigma), which measures the release of lactate dehydrogenase (LDH) by dying cells. Cell viability was estimated as the inverse ratio to LDH release for each experimental condition divided by total LDH release obtained by membrane permeabilization with Triton X-100 (0.01%, 15 min).

RNA Isolation and Reverse Transcriptase (RT)-PCR Analysis—Total cytoplasmic RNA of mouse Schwann cells was extracted using 500 μl of RNAnow-TC extraction kit (Biogentex) according to the manufacturer's protocol. The RT-PCR was performed as follows: cDNA synthesis was carried out in a 20-μl reaction volume containing the total cytoplasmic RNA, 2.5 μM random primer (Roche Molecular Biochemicals SA, Meylan, France), 250 μM dNTPs (Amersham Biosciences, Saclay, France), 5 mM dithiothreitol, 20 units of RNase inhibitor (Promega, Paris, France), and 200 units of SuperScript™II reverse transcriptase (Invitrogen, Cergy Pontoise, France). After incubation overnight at 37 °C, samples were heated to 95 °C for 5 min and kept at −80 °C. 4 μl

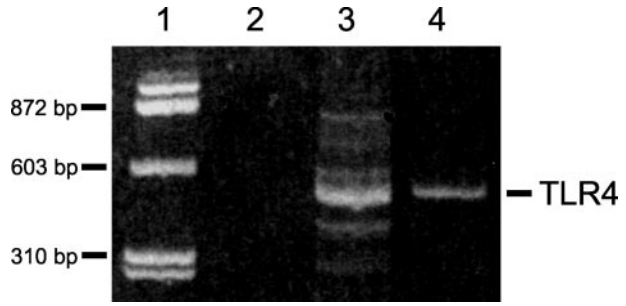

FIG. 1. **Mouse Schwann cells express TLR4 mRNA.** Dorsal root ganglia were excised from organotypic cultures to remove the neuronal population. Total cytoplasmic RNA was extracted as described under "Experimental Procedures" and used for RT-PCR. Primary glial cells from mouse brain were used as a positive control. The amplicons were revealed by UV illumination using ethidium bromide. The selected primer generated a single PCR product of 459 bp for TLR4. *Lane 1*, ϕX174; *lane 2*, water; *lane 3*, brain glial cells; *lane 4*, Schwann cells.

of cDNA were amplified in a final reaction volume of 50 μl consisting of 1× PCR buffer (Qiagen, Courtaboeuf, France) supplemented with a 50 μM concentration of each dNTP, a 0.2 μM concentration of each 5′ and 3′ specific primers, 1.5 mM $MgCl_2$, and 2.5 units of *Taq* DNA polymerase (Qiagen, Courtaboeuf, France). Primer sequences were designed from *Mus musculus* P2X$_7$ receptor sequence (GenBank™ accession number NM011027) and purchased from Genset (Paris, France). Primer sequences were: sense P2X$_7$, 5′-CACATTTGGATGGTGGACCA-3′ and antisense P2X$_7$, 5′-ACTTGAAGCCACTGTACTGC-3′. Primer sequences were designed from *M. musculus* TLR4 sequence (GenBank™ accession number NM021297) and purchased from Genset (Paris, France). Primer sequences were: sense TLR4, 5′-GAATTAAGCTCCATGAACTG-3′ and antisense TLR4, 5′-TCTAGATAGCTGAGACTTGG-3′. The β_2-microglobulin ($\beta_2$mgl) was used as an internal control and was detected using the following primers: sense $\beta_2$mgl, 5′-TGACCGGCTTGTATGCTATC-3′ and antisense $\beta_2$mgl, 5′-CAGTGTGAGCCAGGATATAG-3′. PCR was performed in a Mastercycler personal (Eppendorf France, Le Pecq, France) with the following parameters: denaturation at 94 °C, annealing at 61 °C for P2X$_7$, 60 °C for TLR4, 65 °C for $\beta_2$mgl, and primer extension at 72 °C for 1 min each step (35 cycles for P2X$_7$, 30 cycles for TLR4, 29 cycles for $\beta_2$mgl). The PCR products were separated by 13% acryl/bisacrylamide gel electrophoresis. The amplicons were revealed by UV illumination using ethidium bromide. The incorporation of 1 μCi of [α-^{32}P]dCTP (3000 Ci/mmol, Amersham Biosciences, Les Ullis, France) during PCR allowed for the detection of the amplified product using a PhosphorImager screen (Amersham Biosciences, Bondoufle, France). The signal intensities of RT-PCR products were quantified by calculating the integrated volume of the band with a computing laser densitometer equipped with ImageQuant Software (Amersham Biosciences) normalized to the values for $\beta_2$mgl for each experiment. The selected primers generated a predicted single PCR product of 556 bp for P2X$_7$, 459 bp for TLR4, and 234 bp for $\beta_2$mgl.

Statistical Analysis—Results are expressed as mean ± S.E. Data were submitted to a normality test, and significance was tested by means of Student's paired *t* test and assessed at $p < 0.05$. When mentionned, data were analyzed by a one-way analysis of variance followed by Dunnett's method and assessed at $p < 0.05$.

RESULTS

Production of IL-1β by Mouse Schwann Cells in Organotypic Cultures—Because LPS is described to act specifically through TLR4, we investigated first whether cultured mouse Schwann cells were expressing these receptors by looking at the mRNA level. As shown in Fig. 1, cultured Schwann cells expressed constitutively TLR4 mRNA. Then we studied the effectiveness of a co-treatment with LPS and ATP to induce the production of extracellular IL-1β by investigating the time of ATP treatment required to obtain a steady state level of IL-1β production. Organotypic cultures were primed with LPS during 6 h and with ATP during the last 5, 10, 15, 30, or 45 min of LPS priming. Fig. 2 shows that 5 min of stimulation by ATP led to the production of IL-1β around detection limits. The production of IL-1β became really appreciable after 10 min of stimulation by ATP and increased for longer stimulation to plateau after 30

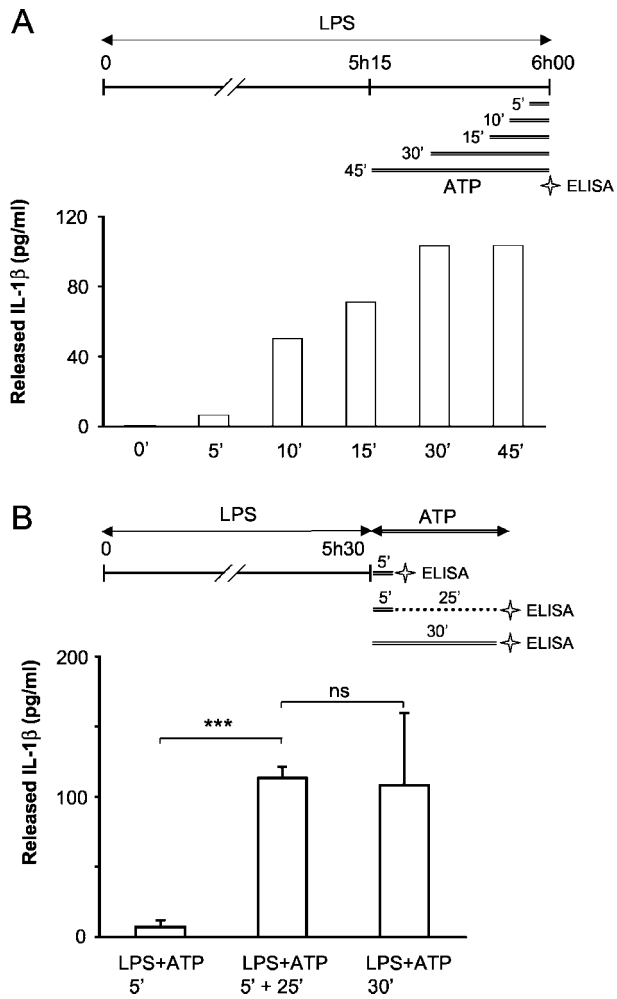

FIG. 2. **Production of IL-1b by organotypic cultures of mouse Schwann cells.** Organotypic cultures were stimulated with LPS (10 μg/ml, 6 h) and with ATP (5 mM) during the indicated times. Extracellular media were collected, and IL-1β was measured by ELISA. *A*, the experimental protocol is described in the *upper panel*. Release of IL-1β became sizeable after 5 min of ATP stimulation and reached a plateau after 30 min. This time course is representative of profiles obtained in three different experiments. *B*, the experimental protocol is described in the *upper panel*. Extracellular medium was collected after 5 or 30 min of ATP stimulation or after 5 min of ATP stimulation followed by a washout of ATP and an incubation in a control medium during 25 min. Note that the concentration of IL-1β produced by a challenge with ATP of 5 min was not significantly different from that produced by 30 min ($[\text{IL-1}\beta]_{5\ \text{min}+25\ \text{min}} = 113 \pm 7.8$ pg/ml, $n = 3$; $[\text{IL-1}\beta]_{30\ \text{min}} = 108 \pm 51.5$ pg/ml, $n = 5$; $p > 0.05$). Each *bar* represents the mean ± S.E.

min. However, as shown in Fig. 2*B*, when culture media were collected 25 min after a brief application of ATP (5 min), the concentration of IL-1β was not significantly different from that produced by 30 min of stimulation by ATP. This result suggests that if 5 min of stimulation of the P2X$_7$ receptor is long enough to trigger IL-1β processing, mechanisms leading to the release of IL-1β need at least 10 min to yield extracellular detectable amounts of the cytokine.

IL-1β Synthesis by Schwann Cells Requires LPS Priming but Not P2X$_7$ Stimulation—Having established the experimental conditions of a sizeable and reproducible production of IL-1β, we then studied the effectiveness of LPS and/or purinergic stimulation to induce the intracellular synthesis of IL-1β. Cultures were either treated with ATP alone for 30 min or primed by LPS for 6 h and treated with ATP during the last 30 min of the protocol. Intracellular IL-1β was assayed in cell lysates by ELISA. Fig. 3*A* shows that intracellular IL-1β was barely detectable in control conditions or after the sole stimulation by

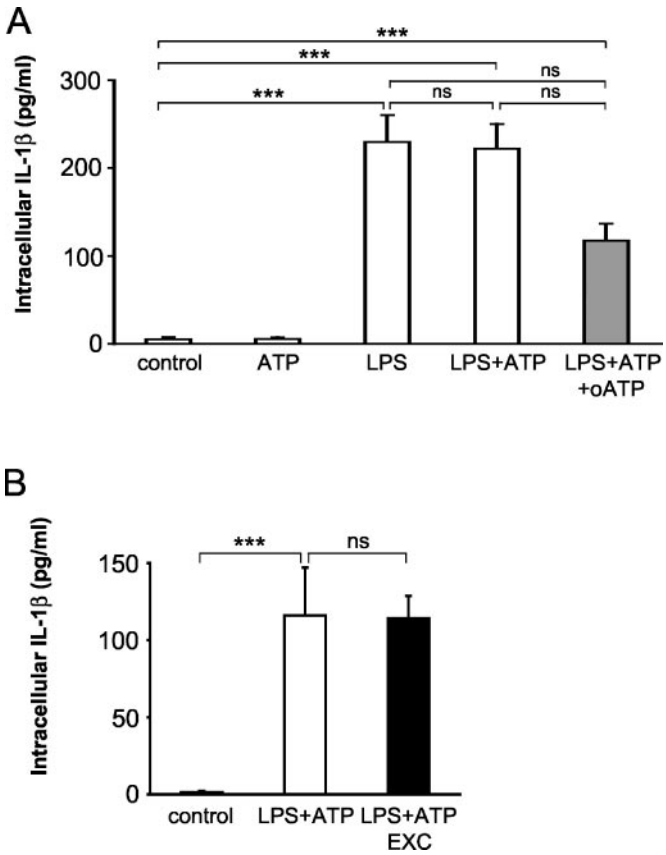

FIG. 3. **LPS stimulates the synthesis of IL-1β in Schwann cells.** Cultured dorsal root ganglia were stimulated with LPS (10 μg/ml, 6 h) and/or with ATP (5 mM, 30 min). Intracellular IL-1β contents were measured by ELISA performed on cell lysates. *A*, LPS triggered the synthesis of IL-1β ($[IL\text{-}1\beta]_{control}$ = 5.2 ± 2.6 pg/ml, n = 17; $[IL\text{-}1\beta]_{LPS}$ = 229.7 ± 30.4 pg/ml, n = 16; $p < 0.001$), whereas ATP did not ($[IL\text{-}1\beta]_{ATP}$ = 5.8 ± 1.8 pg/ml, n = 8). The co-treatment with LPS and ATP did not significantly modify IL-1β synthesis induced by LPS alone ($[IL\text{-}1\beta]_{LPS}$ = 229.7 ± 30.4 pg/ml, n = 16; $[IL\text{-}1\beta]_{LPS+ATP}$ = 221.8 ± 28.3 pg/ml, n = 18; $p > 0.05$). oATP (a specific P2X$_7$ antagonist, 300 μM, 90 min) did not affect IL-1β synthesis induced by the co-treatment with LPS and ATP ($[IL\text{-}1\beta]_{LPS+ATP}$ = 221.8 ± 28.3 pg/ml, n = 18; $[IL\text{-}1\beta]_{LPS+ATP+oATP}$ = 117.9 ± 18.9 pg/ml, n = 7; $p > 0.05$) or by LPS alone ($[IL\text{-}1\beta]_{LPS}$ = 229.7 ± 30.4 pg/ml, n = 16; $[IL\text{-}1\beta]_{LPS+ATP+oATP}$ = 117.9 ± 18.9 pg/ml, n = 7; $p > 0.05$). *B*, excision of dorsal root ganglia from organotypic cultures before the co-treatment LPS + ATP did not change significantly IL-1β synthesis by remaining Schwann cells ($[IL\text{-}1\beta]_{LPS+ATP}$ = 116 ± 31 pg/ml; $[IL\text{-}1\beta]_{LPS+ATP/EXC}$ = 114 ± 14.5 pg/ml, $p > 0.05$). Each *bar* represents the mean ± S.E. of four different experiments (***, $p < 0.001$).

ATP. In contrast, LPS priming of cultures for 6 h led to the synthesis of noticeable amounts of IL-1β. Intracellular IL-1β synthesis induced by LPS priming was not significantly altered by neither a co-treatment with ATP nor the addition of oATP, a potent P2X$_7$ antagonist. As organotypic cultures of dorsal root ganglia contain neurons and Schwann cells, we were led to consider which cell type was synthesizing IL-1β. Therefore we excised dorsal root ganglia to remove the neuronal population before assaying intracellular IL-1β content. In these conditions, IL-1β content of restricted Schwann cells lysate did not significantly differ from IL-1β content of total cells lysate (Fig. 3*B*). These results suggest first, that intracellular IL-1β measured in organotypic cultures was mainly, if not entirely, synthesized by Schwann cells and second, that Schwann cells did synthesize IL-1β only when challenged by an immune stimulus.

Cultured Schwann Cells Release IL-1β When co-stimulated by LPS and ATP—We studied the release of IL-1β by dorsal root ganglia cultures in the same experimental conditions than for intracellular synthesis of IL-1β. In control conditions, IL-1β was barely detectable in culture medium (Fig. 4*A*). LPS priming or ATP stimulation alone did not induce any noticeable release of IL-1β in the culture medium, whereas a co-treatment with LPS and ATP triggered a significant release of IL-1β, which was abolished by pretreatment with oATP. Similar results were obtained in the absence of neurons (Fig. 4*B*).

Pro- and Mature IL-1β Are Released by Schwann Cells—Western blot analysis of intracellular lysates and extracellular media was performed to investigate the production and the release processes of the pro-IL-1β (34–35 kDa) and the mature IL-1β (17 kDa) by dorsal root ganglia cultures. Fig. 5 shows that LPS priming induced the synthesis of pro-IL1β (Fig. 5*A*) but did not induce detectable levels of intracellular (Fig. 5*A*) and extracellular mature IL-1β (Fig. 5*B*). The co-treatment with ATP and LPS did not alter the synthesis of pro-IL-1β, but led to the production of extracellular mature IL-1β (Fig. 5*B*). When Schwann cells were co-treated with LPS and ATP in a potassium-free extracellular medium, mature IL-1β became detectable in intracellular lysates (Fig. 5*A*), and the production of extracellular IL-1β was clearly enhanced (Fig. 5*B*). The pretreatment with oATP (300 μM, 90 min) blocked the maturation of intracellular pro-IL-1β (Fig. 5*A*) and the production of extracellular mature Il-1β (Fig. 5*B*). These results suggest that LPS triggered the synthesis of pro-IL-1β and that ATP, most likely through P2X$_7$ activation, induced not only the processing of pro-IL-1β into mature IL-1β, but also its release.

The Processing of IL-1β by Schwann Cells Needs the Activation of ICE—To assess the involvement of ICE in the processing mechanisms of IL-1β following LPS or LPS + ATP treatments, cultured Schwann cells were primed by LPS, co-treated by ATP with or without an ICE-specific inhibitor, YVAD-CHO (100 μM, 6 h). The addition of YVAD-CHO blocked by more than 95% the production of extracellular IL-1β (Fig. 6*A*). Western blot analysis revealed that YVAD-CHO did not block neither the intracellular synthesis of pro-IL-1β nor its release when co-treated with LPS and ATP (Fig. 6*B*). The activation of ICE was revealed in cultured Schwann cells, using a caspase substrate, FITC-VAD-FMK (10 μM), which fluoresced when cleaved by activated caspases. Fig. 6*C* shows that ATP on its own clearly activated intracellular Schwann cells caspases and that this activation was not altered by LPS priming. The addition of YVAD-CHO strongly reduced the fluorescence, *i.e.* the activation of ICE. When potassium efflux was decreased in high external potassium (90 mM, see below), the activation of ICE was strongly reduced (Fig. 6*D*). Taken as a whole, these results suggest that ATP, through P2X$_7$ receptors and associated potassium fluxes, activates ICE which converts pro-IL-1β into mature IL-1β.

The Production of Extracellular IL-1β Is Modulated by Potassium Fluxes—As shown in Fig. 5, priming by LPS and co-stimulation with ATP in potassium-free conditions increased the maturation of intracellular IL-1β and the production of extracellular IL-1β. This prompted us to investigate whether potassium fluxes were involved in the P2X$_7$ mediated activation of ICE, and what was the relative contribution of large conductance calcium-activated potassium channels (BK channels), one of the two pathways for potassium ions gated by ATP stimulation of P2X$_7$ receptors (28).

To investigate the first issue, we modified potassium gradients by decreasing (from 5 to 0 mM) or increasing (from 5 to 90 mM) the concentration of extracellular potassium. The rationale for these experiments was the following: a potassium-free external solution will favor the outward flux of potassium ions, whereas increasing external potassium to 90 mM would

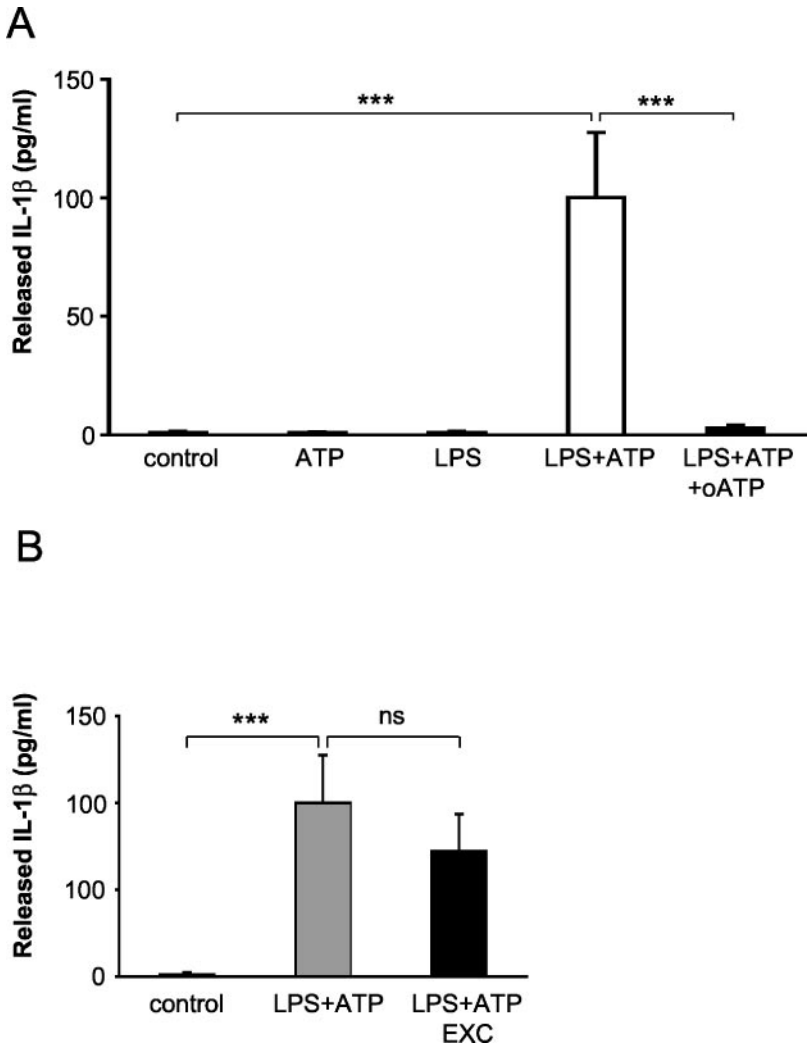

FIG. 4. **P2X$_7$ stimulation induces release of IL-1β by LPS-primed Schwann cells.** Cultured dorsal root ganglia were stimulated with LPS (10 μg/ml, 6 h) and/or with ATP (5 mM, 30 min), and released IL1-β was measured by ELISA performed on extracellular medium. *A*, ATP challenge induced IL-1β release only in LPS-primed cells ($[IL\text{-}1\beta]_{control}$ = 1.21 ± 0.56 pg/ml, n = 8; $[IL\text{-}1\beta]_{LPS}$ = 1.13 ± 0.52 pg/ml, n = 7; $[IL\text{-}1\beta]_{ATP}$ = 1.14 ± 0.23 pg/ml, n = 7; $[IL\text{-}1\beta]_{LPS+ATP}$ = 100.1 ± 27.4 pg/ml, n = 8; $p < 0.001$). oATP (300 μM, 90 min) completely blocked the release of IL-1β ($[IL\text{-}1\beta]_{LPS+ATP+oATP}$ = 3.03 ± 2.94 pg/ml, n = 6; $p < 0.001$). *B*, excision of dorsal root ganglia from organotypic cultures before the cotreatment LPS + ATP did not modify significantly IL-1β release by the remaining Schwann cells ($[IL\text{-}1\beta]_{control}$ = 1.21 ± 0.56 pg/ml, n = 8; $[IL\text{-}1\beta]_{LPS+ATP}$ = 100.1 ± 27.4 pg/ml, n = 8; $[IL\text{-}1\beta]_{LPS+ATP/EXC}$ = 72.3 ± 42.4 pg/ml, n = 4; $p > 0.05$). Each *bar* represents the mean ± S.E.

strongly reduce it. Fig. 7 shows that high external potassium (90 mM) reduced by more than 90% the production of extracellular IL-1β (Fig. 7*A*), whereas external potassium-free solution strongly potentiated (more than 540%) the production of extracellular IL-1β (Fig. 7*B*). These results are in good agreement with the potentiation of ICE activity when the outward flux of potassium is enhanced, the reduction of the outward flux of potassium leading to opposite effects.

Then we looked to the relative contribution of BK channels in IL-1β maturation and release by using charybdotoxin (ChTX) and tetraethylammonium (TEA), two well known BK channels blockers. In the majority of the cases (four experiments out of six), ChTX (100 nM) or TEA (5 mM) reduced the amount of extracellular IL-1β (respectively by about 70 and 75%, Fig 7*C*). In other cases (two experiments out of six, data not shown) TEA had no significant effects, whereas ChTX increased (by about 3.5-fold) the production of extracellular IL-1β (see "Discussion"). Taken together, these results indicate first that potassium fluxes effectively regulate the production of mature IL-1β and second that BK channels modulate Schwann cells ability to produce IL-1β.

The Release of Extracellular IL-1β Is Not Due to ATP-induced Cell Death—ATP-induced cell death is an important issue to investigate, since cytolysis has been proposed as a general mechanism for IL-1β release. Cell viability was assessed by measuring the release of LDH. For all treatments, cell lysis in organotypic culture represented at most 4–5% (Fig. 8). It seems, therefore, very unlikely that a significant amount of extracellular IL-1β would due to the release from dying cells in our experiments.

LPS Priming Does Not Affect P2X$_7$ Receptors and Associated Currents in Mouse Schwann Cells—Having shown that at least one conductance-activated downstream P2X$_7$ stimulation (BK channels) was modulating the production and the release of extracellular IL-1β, we looked for possible effects of LPS priming *per se* on P2X$_7$ receptors and/or the associated conductances. To investigate these issues, we first looked at the expression of P2X$_7$ mRNA in unstimulated cell cultures and in cell cultures primed by LPS for 6 h. As shown in Fig. 9*A*, *panel a*, cultured Schwann cells constitutively expressed P2X$_7$ mRNA in control conditions. The specificity of the amplicon was determined by using *Pst*I restriction enzyme. The expression of P2X$_7$ mRNA was not significantly modified by LPS priming for 6 h (Fig. 9*A*, *panel b*). Then we looked at the P2X$_7$-activated currents (I_{ATP}). LPS priming did not alter the time course of I_{ATP} (Fig. 9*B*, *panels a* and *b*) nor did it modify significantly the mean value of the peak current amplitude of I_{ATP} (Fig. 9*C*). The current-voltage relations for I_{ATP} in control conditions and after LPS priming had the same reversal potential and a similar voltage dependence (Fig. 9*C*). Taken together, these results strongly suggest that LPS did not modify either the expression of the mRNA or the electrophysiological properties of P2X$_7$ receptors.

DISCUSSION

The overall aim of this study was to investigate how P2X$_7$ receptors and their associated conductances expressed by

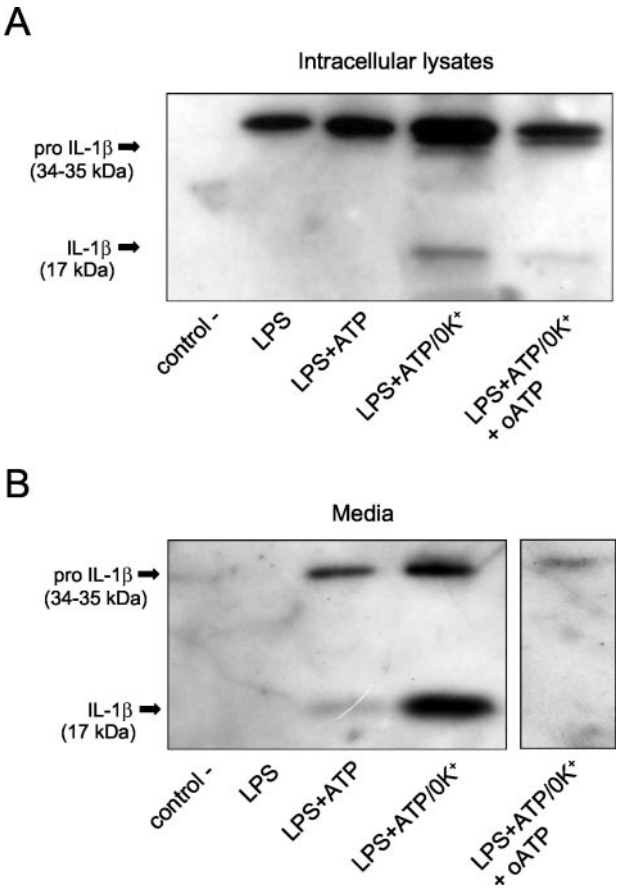

FIG. 5. **P2X$_7$ receptor activation stimulates IL-1β maturation.** Cultured Schwann cells were stimulated with LPS (10 μg/ml, 6 h) and/or with ATP (5 mM, 30 min). Extracellular medium and cell lysates were collected, and forms of IL-1β expressed were determined by Western blot analysis using an antibody reached against pro-IL-1β and mature IL-1β. *A*, intracellular contents on pro-IL1β (34–35 kDa) and mature IL-1β (17 kDa). No mature IL-1β was detectable excepted when the co-treatment LPS + ATP was carried out in extracellular potassium-free medium. oATP (300 μM, 90 min) blocked this production of intracellular mature IL-1β. *B*, extracellular contents of pro-IL1β and mature IL-1β. Pro- and mature IL-1β were found in the extracellular medium. Extracellular potassium-free medium potentiated the release of mature IL-1β. oATP strongly reduced the release of pro-Il-1β and totally blocked the release of the mature form. Each condition was performed on triplicate cultures.

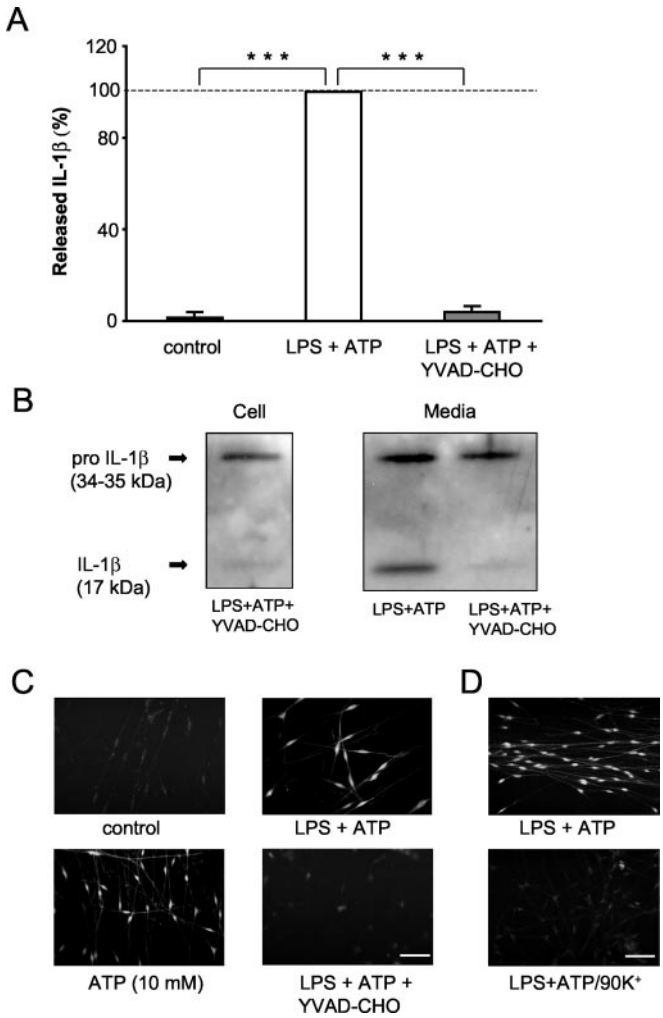

FIG. 6. **ATP-stimulated maturation and release of IL-1β needs the activation of ICE.** Cultured Schwann cells were stimulated with LPS (10 μg/ml, 6 h) with or without ATP (5 mM, 30 min). ICE activity was specifically inhibited by YVAD-CHO (100 nM, 6 h). *A*, ELISA measurements of released IL-1β. Addition of YVAD-CHO blocked, almost entirely, the production of extracellular IL-1β triggered by LPS + ATP ($IL\text{-}1\beta_{control}$ = 1.3 ± 1.8%; $IL\text{-}1\beta_{LPS+ATP}$ = 100%; $IL\text{-}1\beta_{LPS+ATP+YVAD\text{-}CHO}$ = 3.9 ± 3.5%). Each column represents the mean ± S.E. of three different experiments. *B*, Western blot analysis of intracellular and extracellular IL-1β contents. YVAD-CHO blocked the production of mature IL-1β, both in the intracellular and the extracellular compartments. Each condition was performed on duplicate cultures. *C* and *D*, *in situ* detection of caspases activity. Caspase activity was revealed by FITC-VAD-FMK (10 μM) assay. The activation of caspase activity triggered by ATP (10 mM, 30 min) was blocked by YVAD-CHO, suggesting that ATP via the P2X$_7$ receptor specifically activated ICE (*C*). The decrease of the driving force for potassium efflux produced by high external potassium (90 mM) strongly reduced the activation of ICE (*D*). *Scale bar*: 100 μm.

mammalian Schwann cells can regulate IL-1β processing and, therefore, contribute to their immune functions. From the results presented here, one can draw a general scheme of coordinated events. The immune challenge of Schwann cells by LPS, through interaction with surface membrane TLR4, triggers the intracellular synthesis of pro-IL-1β. The processing of pro-IL-1β, the inactive precursor, into mature IL-1β needs a co-stimulation by exogenous ATP. The mechanism by which extracellular ATP activates IL-1β maturation involves P2X$_7$ receptors whose stimulation activates ICE, which converts pro-Il-1β to mature IL-1β. The activation of ICE is potentiated by a depletion in the intracellular concentration of potassium. This depletion is mediated by potassium efflux through the P2X$_7$ receptor itself, but also by a calcium-activated potassium conductance, which is gated downstream of the activation of the P2X$_7$ receptor (26). The release of IL-1β by LPS-primed Schwann cells is also dependent of the stimulation of P2X$_7$ receptors but does not involve cell death. Taken together these results demonstrate, for the first time in mammalian Schwann cells, that the P2X$_7$ receptor is needed to process and release IL-1β and bring new insights on links between the P2X$_7$ receptor, potassium fluxes through different pathways and IL-1β processing.

Toll-like receptors play a central role in triggering innate immune responses. They recognize specific molecular patterns carried by different pathogens termed pathogen-associated molecular patterns. Up to now, 10 different TLRs have been identified, each one being linked to particular pathogen-associated molecular patterns (30). TLR4 is specifically activated by the endotoxin LPS, a major component of the cell wall of Gram-negative bacteria, and its stimulation leads to the activation of the transcription factor NF-κB, which regulates the expression of pro-IL-1β (31) as well as other several immune response genes (32). In the present work, we show for the first time that cultured mouse Schwann cells expressed constitutively TLR4

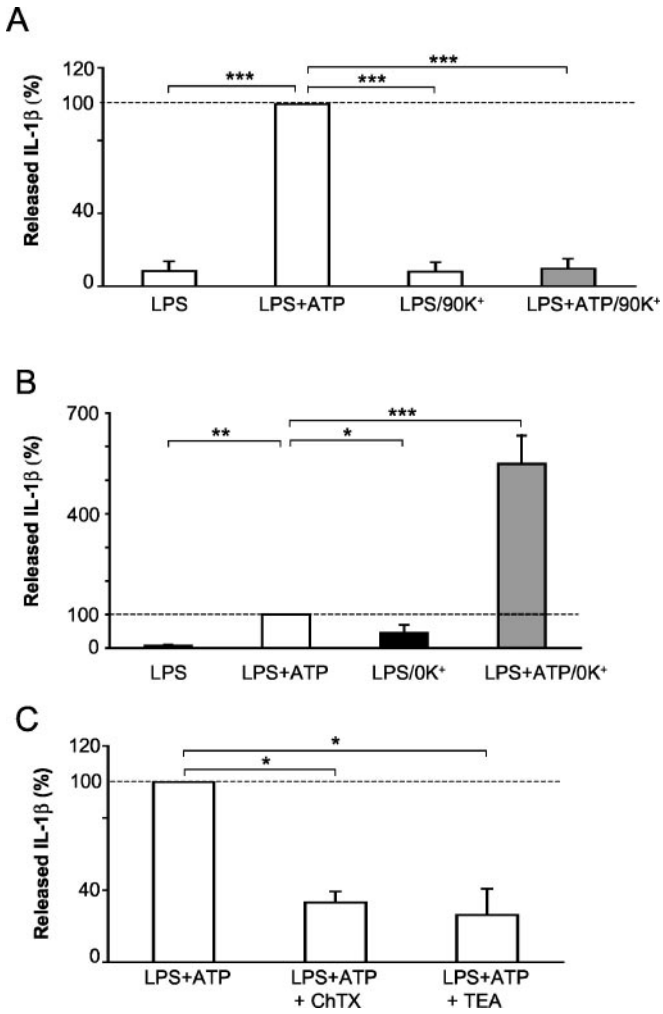

FIG. 7. **Potassium efflux is involved in maturation and release of IL-1β by Schwann cells.** Cultured Schwann cells were stimulated with LPS (10 μg/ml, 6 h) with or without ATP (5 mM, 30 min). Potassium fluxes were altered during ATP stimulation by modifying the driving force for potassium. *A*, high external potassium ($[K^+]$ = 90 mM), which strongly reduce the efflux of potassium ions, blocked by more than 90% the production of extracellular IL-1β ($IL\text{-}1\beta_{LPS+ATP}$ = 100%, n = 3; $IL\text{-}1\beta_{LPS}$ = 9.3 ± 0.3%, n = 3, $p < 0.001$; $IL\text{-}1\beta_{LPS/90\ K^+}$ = 8.7 ± 0.3%, n = 3, $p < 0.001$; $IL\text{-}1\beta_{LPS+ATP/90\ K^+}$ = 9.6 ± 0.4%, n = 3, $p < 0.001$). *B*, external potassium-free medium, which favors the efflux of potassium ions, enhanced by about 5.5-fold the production of extracellular IL-1β ($IL\text{-}1\beta_{LPS+ATP}$ = 100%, n = 5; $IL\text{-}1\beta_{LPS}$ = 6.6 ± 7.2%, n = 5, $p < 0.01$; $IL\text{-}1\beta_{LPS/0\ K^+}$ = 45 ± 39%, n = 3, $p < 0.05$; $IL\text{-}1\beta_{LPS+ATP/0\ K^+}$ = 548.7 ± 188.7%, n = 5, $p < 0.001$). *C*, ChTX (100 nM) and TEA (5 mM), two blockers of calcium-activated potassium channels, reduced the production of extracellular IL-1β ($IL\text{-}1\beta_{LPS+ATP}$ = 100%; $IL\text{-}1\beta_{LPS+ATP+ChTX}$ = 32.9 ± 12.6%, $p < 0.05$; $IL\text{-}1\beta_{LPS+ATP+TEA}$ = 26.2 ± 25%, $p < 0.05$). Each *bar* represents the mean ± S.E. of four experiments. Data were analyzed by a one-way analysis of variance followed by Dunnett's method and assessed at $p < 0.05$ (*, $p < 0.05$; **, $p < 0.01$).

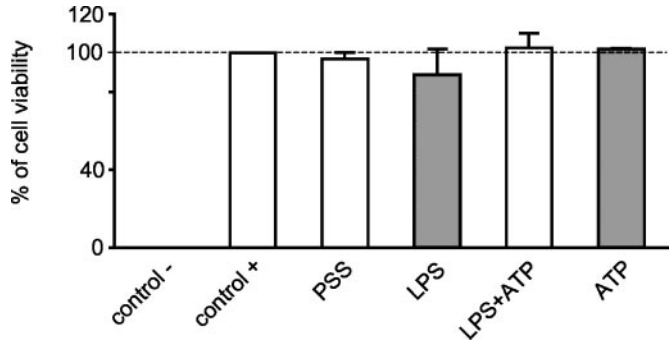

FIG. 8. **Schwann cell viability after LPS and ATP treatments.** Cell viability was determined by LDH release assay. Negative control of cell viability was obtained by incubating cells with Triton X-100 (0.01%, 15 min), and positive control was obtained by incubating cells in control culture medium (15 min). Release of LDH, a cytoplasmic enzyme, is representative of cell death. Results of negative and positive control represent, respectively, 0 and 100% of cell viability. Other results are expressed as percent of cell viability. Schwann cell viability was not significantly altered by PSS (96.8 ± 3.3%), LPS (88.9 ± 12.9%), ATP (102 ± 0.3%), or LPS+ATP (102.8 ± 7.3%) treatments. Each *bar* represents the mean ± S.E. of three different experiments.

mRNA, which led to the insertion of functional receptors at the surface membrane. As a result of the stimulation of TLR4 by LPS, mouse Schwann cells synthesize pro-IL-1β but neither process nor release it. Interestingly, cultured Schwann cells also expressed the decay accelerating factor (DAF/CD55) (33) recently suggested to be a part of the multimeric LPS receptor complex (34). Therefore, cultured Schwann cells express membrane receptors and do possess the needed intracellular machinery to trigger an innate immune response to bacterial LPS. This points out the general question of the physiological relevance of this receptor in glial cells of the peripheral nervous system. Clearly, there is not yet a straightforward answer to this question as at first sight the peripheral nervous system could seem protected by the blood nerve barrier from bacterial assaults. However, the peripheral nervous system is not entirely sheltered from bacteria. As a prototypical example, *Mycobacterium leprae*, the causative agent of leprosy, acts specifically on Schwann cells to induce their apoptosis and causes nerve damage. Very interestingly, Schwann cells express TLR2, which seems responsible for the ability of *M. leprae* to induce Schwann cell apoptosis (35). The infancy of the knowledge of the mammalian TLR family as key molecules of the innate immunity probably narrow our vision of the relevance of these molecules in Schwann cells. Thus, it is doubtless that if TLRs are expressed *in vivo* by Schwann cells, they could be regarded as important items of the panoply used by Schwann cells to regulate local inflammatory responses that take place within the peripheral nervous system.

Our results clearly show that the processing of pro-IL-1β into mature IL-1β needs the activation of P2X$_7$ receptors and is most likely related to P2X$_7$-activated potassium fluxes. The involvement of potassium fluxes in the processing of IL-1β was first reported by Perregaux *et al.* (36) when they showed that IL-1β maturation and release by LPS-primed mouse macrophages could be induced by application of nigericin, a selective potassium ionophore. Following this pioneer work, Perregaux and Gabel (15) showed on the same cells that the sustained application of ATP (5 mM, 20 min) was triggering a large efflux of potassium, similar to the effect of nigericin, and that this ATP-induced IL-1β release could be blocked by an excess of potassium in the extracellular medium. Last, Cheneval *et al.* (37) demonstrated that lowering intracellular potassium was triggering the activation of ICE. From these reports emerged the idea that ICE was likely inhibited by physiological concentrations of intracellular potassium, *i.e.* within the range 130–150 mM, whereas a decrease in intracellular potassium relieved this tonic inhibition. In recent work (26) we reported that ATP stimulation of P2X$_7$ receptors in mouse Schwann cells lead to an outward movement of potassium ions by at least two pathways: through the non-selective cationic P2X$_7$ pore itself and through downstream-activated large conductance calcium-activated potassium channels (BK channels), a pathway up to now only reported in mouse Schwann cells. The requirement of ICE activity in the production process of extracellular mature IL-1β and the modulation of its release by alterations of the potassium gradient shown in the present work fit well with a general scheme in which the tonic inhibition of ICE by intra-

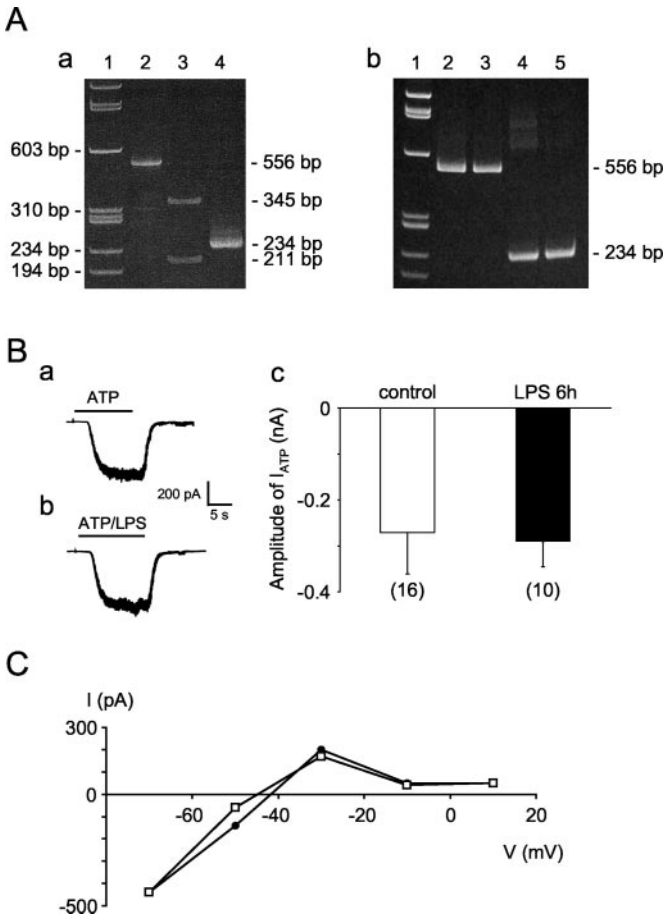

FIG. 9. **Lack of effect of LPS priming on P2X$_7$ mRNA expression and P2X$_7$-mediated responses in Schwann cells.** *A*, dorsal root ganglia were excised from organotypic cultures to remove the neuronal population. Total cytoplasmic RNA was extracted as described under "Experimental Procedures" and used for RT-PCR. *Panel a*, the selected primer generated a single PCR product of 556 bp for P2X$_7$ and 234 bp for $\beta_2$mgl. The specificity of the P2X$_7$ amplicon was determined by digestion with *Pst*I restriction enzyme that yielded two fragments at the predicted sizes, *i.e.* 345 and 211 bp. *Lane 1*, ϕX174; *lane 2*, P2X$_7$; *lane 3*, P2X$_7$/*Pst*I; *lane 4*, $\beta_2$mgl. *b*, LPS priming (6 h) did not induce a significant modification in the P2X$_7$ mRNA expression (control conditions: P2X$_7$/$\beta_2$mgl = 1.23 ± 0.79; LPS treatment: P2X$_7$/$\beta_2$mgl = 1.19 ± 1.01, $p > 0.9$). *Lane 1*, ϕX174; *lane 2*, control P2X$_7$; *lane 3*, LPS/P2X$_7$; *lane 4*, control $\beta_2$mgl; *lane 5*, LPS/$\beta_2$mgl. One representative experiment out of four separate experiments with different cultures is shown. *B*, Schwann cell currents induced by ATP (10 mM) in control conditions or after LPS priming (10 μg/ml, 6 h). Schwann cells were held at −70 mV and recorded in the whole-cell configuration of the patch clamp technique. Note that LPS priming did not seem alter the time course of I_{ATP} (*panel a*: control; *panel b*: LPS priming) and the mean value amplitude of I_{ATP} (*panel c*: I_{ATP} = −271 ± 90 pA, n = 16 taken from Ref. 29; $I_{ATP+LPS}$ = −290 ± 55 pA, n = 10; $p > 0.05$). *C*, the current-voltage relationship for I_{ATP} in control conditions and after LPS priming had the same reversal potential and a similar voltage dependence.

cellular potassium ions in basal conditions would be relieved by a P2X$_7$-activated efflux of potassium ions. The modulation of the production of extracellular IL-1β by BK channels blockers confirmed the involvement of this pathway in IL-1β processing. As expected, in most cases (four out of six), the specific blockade of this conductance either by TEA or by ChTX reduced noticeably the production of extracellular IL-1β. Surprisingly, in some experiments (two out of six), the production of extracellular IL-1β was not significantly affected by TEA and potentiated by ChTX. Such potentiation could be related to differences in the relative level of expression of BK channels (see below).

As shown by Western blot experiments (see Fig. 5), the involvement of P2X$_7$ receptor does not seem restricted to the processing of pro-IL-1β into mature IL-1β but also extends to the release of the cytokine. Among the "classical" P2X$_7$-associated responses, cell death often comes first. P2X$_7$ receptor-mediated cytotoxicity has been described for sustained stimulation by high concentration of extracellular ATP (9) and associated to the large dilatation of the non selective cationic pore, up to a molecular cutoff around 900 Da (38). Following this school of thought, and also because of the lack of secretory signal sequence of IL-1β, it is generally considered that the extracellular production of mature IL-1β by immune cells is a side effect of ATP-induced apoptotic and/or necrotic cell death (for review, see Ref. 9). Searching for ATP-mediated Schwann cell death, we found no evidence for such phenomenon in any of our experimental conditions. This confirmed that the release of IL-1β by Schwann cells was not a side effect of cell death. This is quite important, as it indicates first that P2X$_7$ receptors participate also to the release of IL-1β, and second, that non-cytolytic mechanisms of IL-1β release operate in mammalian Schwann cells. In generic macrophages or monocytes, three non-cytolytic mechanisms of Il-1β release have been documented: (i) the exportation by an ATP-binding cassette transporter involving chloride conductances (12), (ii) the secretion by microvesicle shedding (11), and (iii) the exportation by ICE itself (14). Although the present work does not rule out the two last mechanisms, it is likely that the first mechanism is involved in mouse Schwann cells. Indeed, P2X$_7$ receptor activation in those cells is associated with the gating of a chloride conductance that is dependent on intracellular potassium ions (26), and preliminary data show that chloride channel blockers strongly reduce the production of extracellular IL-1β.[2] This could outline an integrated role of P2X$_7$ receptors and associated conductances where BK channels would increased potassium efflux, ICE activation, and the maturation of IL-1β, and chloride conductance would be involved in the release of IL-1β. In this hypothesis, a strong and/or maintained depletion in potassium could impair the functioning of the chloride conductance and eventually decrease the release of IL-1β. Such mechanisms could explain that in some experiments the production of extracellular IL-1β was not significantly affected by TEA and potentiated by ChTX. For those batches of cells, the mean value of extracellular IL-1β released in control conditions was remarkably lower ([IL-1β] = 12.3 ± 2.3 pg/ml compared with [IL-1β] = 100.1 ± 27.4 pg/ml). According to our hypothesis, such low levels of production in control conditions could be explained by a relative increase in the surface density in BK channels. As a likely consequence, the increase in the relative strength of the potassium efflux through this pathway could impair partially the functioning of the chloride conductance activated downstream the P2X$_7$ receptor. Therefore, for those Schwann cells with a higher level of expression of BK channels, the blockade of these channels would restore the production of IL-1β instead of decreasing it. Clearly, this point needs further work to be clarified, but nevertheless this pathway may represents a novel putative target to modulate the ability of Schwann cells to synthesize and release IL-1β.

P2X$_7$ receptors have been reported to activate in microglia the nuclear factor of activated T cells (NFAT) (39) and the nuclear factor-κB (NF-κB) (40), both transcription factors controlling the expression of several cytokines. These pathways open the interesting possibility of a direct purinergic activation of the cytokine network, *i.e.* in the absence of an immune stimulus. However, our results show clearly that the sole stimulation of P2X$_7$ receptors did not induce a detectable synthesis of intracellular pro-IL-1β. Few explanations can be put for-

[2] V. Marty and T. Amédée, unpublished data.

ward. Either the mouse P2X$_7$ receptor is not coupled to the NF-κB pathway, or 30 min of stimulation of the P2X$_7$ receptor was not long enough to induce the activation of the NF-κB pathway (40). Alternatively, and also shown for microglial cells (40), ATP (via the P2X$_7$ receptor) could induce NF-κB p65 homodimers instead of prototypical p50 homodimers and p65/p50 heterodimers in Schwann cells. This remains to be determined, but such a pathway would make sense considering that (i) p65 homodimers bind preferentially to a site of the IL-8 promoter (41), (ii) IL-8, a chemotactic cytokine is produced by Schwann cells during inflammatory processes (42), and (iii) the immune status of Schwann cells is very much dependent on the P2X$_7$ receptor (22).

In conclusion, the P2X$_7$ receptor occupies a privileged position in the immune capabilities of Schwann cells. While being responsible for the production of IL-1β, it acts upstream inflammatory processes and therefore represent an important element in the regulation of the cytokine network. Moreover, its actions within the cytokine network seem modulated at the transcription level, by the network itself. Hence, if LPS alone had no appreciable effect on the expression of P2X$_7$ receptor mRNA in mouse Schwann cells and in macrophages (43), a co-treament LPS with pro-inflammatory cytokines such as IFNγ or TNFα did up-regulate P2X$_7$ receptor mRNA expression (44). Depending on the fate of inflammation, blockade of the P2X$_7$ receptor activity could be beneficial when inflammation is noxious like in the Guillain-Barre syndrome or, on the contrary, whose maintenance could be salutary when inflammation is followed by tissue regeneration like in Wallerian degeneration. As long as there is a lack of *in vivo* studies, the precise role of P2X$_7$ receptors in peripheral neuropathogenesis will remain speculative. However, it is worthy to note that *in vivo*, the production of IL-1α and Il-1β in rats with experimental autoimmune neuritis is located at paranodal regions (24), where actually P2X$_7$ receptors are preferentially expressed (45). More direct evidence of the involvement of P2X$_7$ receptors in the production of extracellular IL-1β has been reported in other tissues. It has been shown *in vivo* that the release of IL-1β by murine peritoneal macrophages (16) and by human circulating blood monocytes (46) was induced by treatment with ATP. The role of the P2X$_7$ receptor in mammalian Schwann cells is certainly not only limited to the production of IL-1β, since by analogy with other glial or immune cells, it could be involved in the production of chemotactic molecules like the monocyte chemoattractant protein-1 (20), other pro-inflammatory cytokines such as TNFα (47), IL-6 (48), IL-18 (49), and other mediators of inflammatory processes (nitric oxide, prostangladins, thromboxans) (for review, see Ref. 22).

Acknowledgments—We thank Laëtitia Malphettes and Magali Koutti for help in Western blot experiments and Estelle Drouillard for her expert technical assistance in dorsal root ganglia cultures. We are also very grateful to Prof. Richard Robitaille and Dr. John E. Sims for reading and helpful comments on this manuscript.

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Maturation and Release of Interleukin-1β by Lipopolysaccharide-primed Mouse Schwann Cells Require the Stimulation of P2X $_7$ Receptors

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J. Biol. Chem. 2003, 278:30732-30740.
doi: 10.1074/jbc.M304534200 originally published online June 9, 2003
