Brief Communications

Up-Regulation of $P2X_4$ Receptors in Spinal Microglia after Peripheral Nerve Injury Mediates BDNF Release and Neuropathic Pain

Lauriane Ulmann,[1,2] Jon P. Hatcher,[3] Jane P. Hughes,[3] Séverine Chaumont,[1,2] Paula J. Green,[3] François Conquet,[4] Gary N. Buell,[4] Alison J. Reeve,[3] Iain P. Chessell,[3] and Francois Rassendren[1,2]

[1]Institut de Génomique Fonctionnelle, Centre National de la Recherche Scientifique, Unité Mixte de Recherche 5203, and [2]INSERM, U661, and Université Montpellier, 1, 2, 34094 Montpellier, France, [3]Pain Research, Neurology and Gastrointestinal Centre of Excellence for Drug Discovery, GlaxoSmithKline, Harlow, Essex CM19 5AW, United Kingdom, and [4]GlaxoWellcome Biomedical Research Institute, Geneva 1228, Switzerland

ATP is a known mediator of inflammatory and neuropathic pain. However, the mechanisms by which specific purinergic receptors contribute to chronic pain states are still poorly characterized. Here, we demonstrate that in response to peripheral nerve injury, $P2X_4$ receptors ($P2X_4R$) are expressed *de novo* by activated microglia in the spinal cord. Using *in vitro* and *in vivo* models, we provide direct evidence that $P2X_4R$ stimulation leads to the release of BDNF from activated microglia and, most likely phosphorylation of the NR1 subunit of NMDA receptors in dorsal horn neurons of the spinal cord. Consistent with these findings, P2X4-deficient mice lack mechanical hyperalgesia induced by peripheral nerve injury and display impaired BDNF signaling in the spinal cord. Furthermore, ATP stimulation is unable to stimulate BDNF release from $P2X_4$-deficient mice microglia in primary cultures. These results indicate that $P2X_4R$ contribute to chronic pain through a central inflammatory pathway. $P2X_4R$ might thus represent a potential therapeutic target to limit microglia-mediated inflammatory responses associated with brain injury and neurodegenerative disorders.

Key words: **$P2X_4$; microglia; BDNF; pain; knock-out; mice**

Introduction

Among the seven P2X receptors, $P2X_4$ displays the most widespread tissue distribution (Khakh and North, 2006; Burnstock, 2007). In the nervous system, $P2X_4$ receptors ($P2X_4R$) are expressed in neurons of different brain regions (Burnstock, 2007). In CA1 pyramidal neurons $P2X_4R$ are expressed postsynaptically (Rubio and Soto, 2001), and are activated during high frequency stimulation and thus participate to synaptic potentiation (Sim et al., 2006). $P2X_4R$ are also expressed within the immune system. In inflammatory lymphocytes and monocytes, $P2X_4R$ mRNA are the most strongly expressed among all P2X receptors (Wang et al., 2004). Functional and pharmacological approaches have also demonstrated the presence of $P2X_4$ channels in peripheral macrophages, where they are often (but not always) associated with other P2XR, mostly $P2X_1$ and $P2X_7$ (Buell et al., 1996; Sim et al., 2007). Similarly, immunohistochemistry has revealed expression of P2X4R in microglial cells recruited after brain or nerve lesions (Tsuda et al., 2003; Zhang et al., 2006). Because of the lack of a highly specific $P2X_4R$ antagonist, deciphering the functional roles of $P2X_4R$ in macrophages and microglia still remains difficult. In one study, antisense knock-down of $P2X_4R$ expression in spinal microglia provided compelling evidence of their functional involvement in tactile allodynia (Tsuda et al., 2003).

In the healthy CNS, microglia are considered to be in a so-called resting state, although constantly surveying the environment (Biber et al., 2007). On rupture of brain homeostasis, microglia rapidly switch to an activated state, characterized by transcriptional and functional remodelling and by the acquisition of an immuno-competent phenotype (Hanisch and Kettenmann, 2007). Purinergic receptors appear to be key players in microglia signaling, both in their resting and activated states (Färber and Kettenmann, 2006). P2Y receptors regulate motility and cellular chemotaxis, two resting microglia processes (Haynes et al., 2006), whereas in the activated state they mediate phagocytosis (Koizumi et al., 2007). As for P2X receptors, immunohistochemistry data has revealed the expression of $P2X_4$ and $P2X_7$ in microglial cells surrounding brain lesions or regions undergoing neurodegenerescence (Parvathenani et al., 2003), where they are likely to promote a local inflammatory response. Indeed, $P2X_7$ receptor activation leads to the secretion of IL-1ß from microglial cell lines and $P2X_7$-deficient mice do not develop mechanical hypersensitivity associated with neuropathic pain (Ferrari et al., 1997; Chessell et al., 2005). Similarly, $P2X_4R$ expressed by activated spinal microglia after peripheral nerve injury promote neuropathic pain (Tsuda et al., 2003).

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Correspondence should be addressed to Francois Rassendren, Institut de Génomique Fonctionnelle, CNRS UMR5203, 141 rue de la Cardonille, 34094 Montpellier, France. E-mail: far@igh.cnrs.fr.

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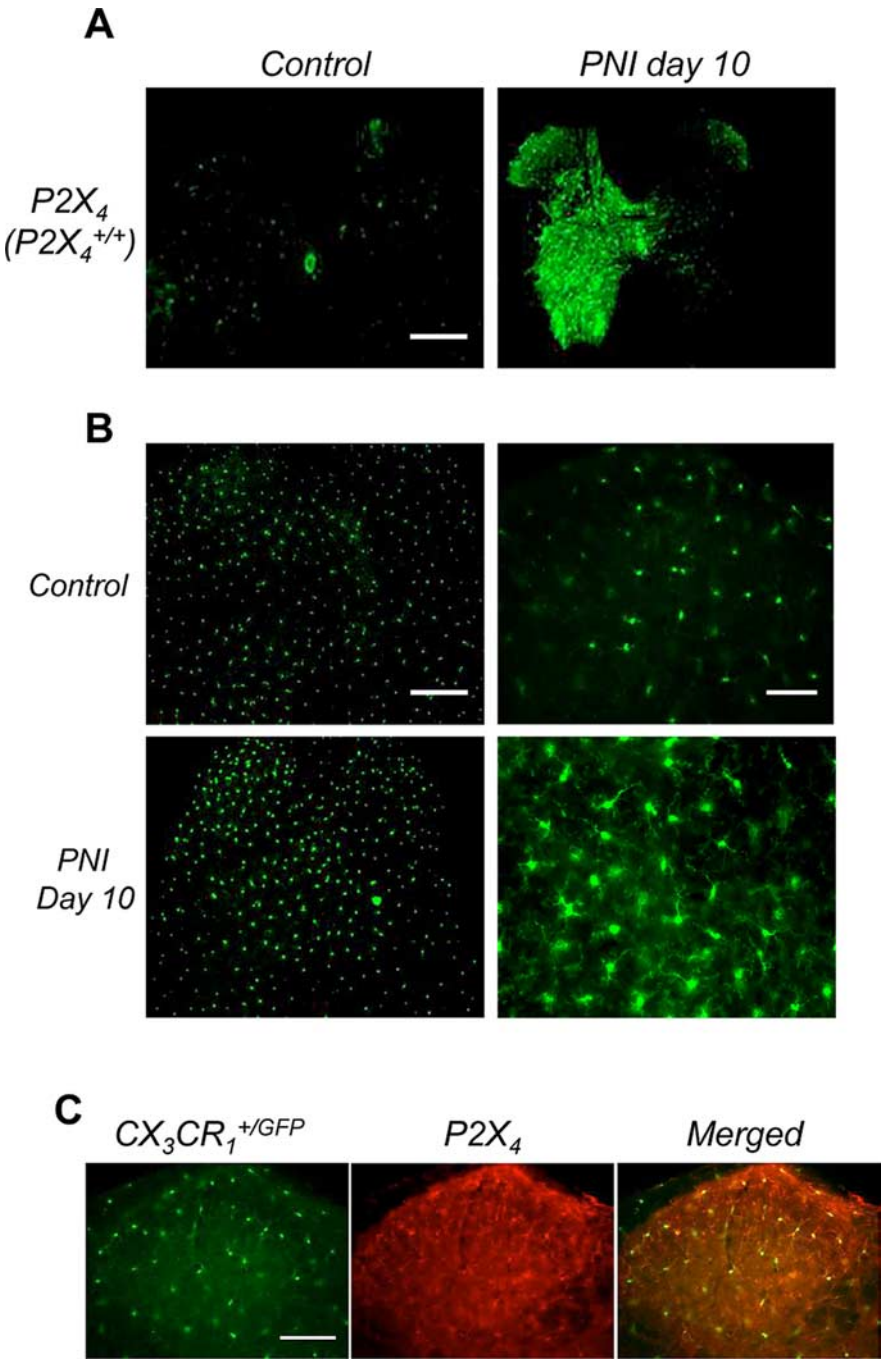

Figure 1. Peripheral nerve injury induces upregulation of $P2X_4R$ in activated spinal microglia. ***A***, Comparison of P2X4 expression in the spinal cord in sham-operated and injured mice. $P2X_4$ immunoreactivity was barely detectable in sham animals whereas 10 d PNI, it was clearly induced ipsilateral to the lesion. Note that $P2X_4$ expression spread to the whole side of the spinal cord because of sensory and motor fiber lesions. Scale bar, 500 μm. ***B***, Morphology of spinal microglia in *CX3CR1* $^{+/GFP}$ mice post-PNI. Compared with sham animals, 10 d post-PNI microglia displayed higher fluorescence intensities, whereas the number of GFP cells was not significantly different. Increase of fluorescence intensity was restricted to the ipsilateral side of the lesion. Scale bar, 500 μm. At higher magnification in the dorsal horn region of the spinal cord (right), post-PNI microglia presented the typical morphological characteristics of the activated state with larger cell bodies and compacted processes compared with microglia from sham animals. Scale bar, 50 μm. ***C***, PNI induces P2X4 expression in activated microglia. In the dorsal horn, P2X4 immunoreactivity colocalized exclusively with microglia-specific eGFP fluorescence in *CX3CR1* $^{+/GFP}$ mice 10 d post-PNI. Scale bar, 100 μm.

In vivo, intrathecal injection of ATP-stimulated microglia causes the development of allodynia within a few hours. This has been attributed to the secretion of BDNF by spinal microglia that in turns reduces the tonic inhibition of lamina I GABAergic interneurons (Coull et al., 2005). The subtype of purinergic receptor responsible for this ATP-induced BDNF secretion by microglia is still unknown because microglia express different types of purinergic receptors among which at least two ($P2X_4$ and $P2X_7$) have been shown to promote neuropathic pain. In this study we used $P2X_4$-deficient mice as an animal model to investigate the potential involvement of $P2X_4R$ in ATP-mediated BDNF microglial secretion and neuropathic pain.

Materials and Methods

Targeting of the $P2X_4$ *gene and generation of mutant mice.* Mice carrying a targeted null mutation of the *$P2X_4$* gene have been described previously (Sim et al., 2006). Briefly, a ß-galactosidase-neomycin cassette was inserted in place of the first coding exon of the *$P2X_4$* gene. In $P2X_4$ knock-out mice, the $P2X_4$ promoter drives β-galactosidase expression. *$P2X_4^{+/-}$* mice were fully backcrossed on the C57BL/6 strain (n >20 generations) and then maintained as separate $P2X_4$ knock-out (*$P2X_4^{-/-}$*) and wild-type (*$P2X_4^{+/+}$*) lines. Mice were housed under a standard 12 h light/dark cycle with food and water available *ad libitum*. Mice used in separate tests were age and sex matched to reduce any variation; age varied between 6 and 12 weeks. All procedures fully complied with French legislation (décret 87-848, October 19, 1987, and order, April 19, 1988), which implement the European directive (86/609/EEC) on research involving laboratory animals, and were performed according to the requirements of GlaxoSmithKline and CNRS ethical standards.

Partial nerve ligation. Cohorts of 15 male *$P2X_4^{-/-}$* and *$P2X_4^{+/+}$* mice were used for this study. On day 0, before surgery, mice were tested as described below to establish baseline thresholds. All mice underwent surgery to partially ligate the sciatic nerve using a method based on that described by (Seltzer et al., 1990). Mice were anesthetized with isoflurane and ~1 cm of their left sciatic nerve was exposed by blunt dissection at mid-thigh. A suture was then passed through the dorsal third of the nerve and tied tightly. The incision was sutured and the mice were left for 3 d before testing started.

Measurement of mechanical hyperalgesia. To assess mechanical hyperalgesia, mice were tested for withdrawal thresholds using an analgesimeter on days 3, 7, 10, 14 and 24 post-operation, as previously described (Chessell et al., 2005). Both ipsilateral and contralateral withdrawal thresholds were measured and expressed as ipsilateral/contralateral ratios. Results were analyzed using two-way ANOVA in Statistica (Statsoft Inc.) with genotype and days postsurgery being used as independent variables. Follow-up analysis was performed using Duncan's test and $p < 0.05$ was considered significant.

Microglia cultures. Primary microglial cells were isolated from 1 d postnatal mice. Briefly, cortices were homogenized by mechanical dissociation and mixed glial cells were plated for two weeks in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (Biowest) and 1% penicillin/streptomycin. Cultures were flushed and

purified microglial cells were collected before centrifugation and plating. Pure (>95%) microglial cultures were used within 2 d.

Immunocytochemistry and immunohistochemistry. The following antibodies were purchased from commercial sources: rabbit-anti-BDNF 1/100 (Santa Cruz) and rabbit-anti-BDNF 1/100 (Abcam), mouse anti-β-galactosidase 1/50,000 (Promega), rabbit anti-phospho-NR1 (Ser896) 1/1,000 (Upstate), rabbit anti-Iba1 1/1,000 (Wako). $P2X_4$ antibody (1/500) was produced in the laboratory using the 19 carboxyterminal residues of mouse $P2X_4$ as immunogenic peptide. Tissues were fixed through a transcardiac perfusion of 4% paraformaldehyde in PBS and post-fixed overnight. Vibratome sections (50 μm) were permeabilized using 0.1% Triton X-100 in PBS, nonspecific sites were blocked with 10% FCS, 0.1% Triton X-100 in PBS for 30 min at room temperature and incubated overnight at 4°C with primary antibody. After three washes in PBS, sections were incubated for 2 h with secondary species-specific antibody [goat-anti rabbit Alexa488, donkey-anti mouse Alexa594 secondary antibody (Invitrogen)]. Sections were mounted and viewed with a Leica DMRA2 fluorescent microscope. Images were acquired using a cool-snap HQ (PhotoMetrics) digital camera controlled by the Metaview software suite. For immunocytochemistry, cells plated on coverslips were fixed for 10 min with 4% paraformaldehyde in PBS. Immunolabeling and image acquisition were performed as described above.

COS-7 cell transfection. COS-7 cells (ATCC # CRL-1651) were grown in DMEM supplemented with 10% fetal calf serum and 1% penicillin/streptomycin. One microgram of cDNA encoding mouse $P2X_4$ and/or BDNF-GFP were transfected at a ratio of 1/3 using lipofectAMINE 2000 (Invitrogen) according to the manufacturer's instructions.

Western blot. Microglia and COS-7 cells were homogenized in a lysis buffer containing 100 mM NaCl, 5 mM EDTA, 1% Triton X-100, Complete protease inhibitor mixture (Roche), and 20 mM Hepes, pH 7.4. Lysates were clarified by centrifugation and protein concentration determined using a protein assay kit (Bio-Rad). Proteins were separated on reducing 8% SDS-PAGE, and transferred to a nitrocellulose membrane. The membrane was blocked with 5% nonfat dry milk/0.5% Tween20 in Tris buffer saline (TBST) overnight at 4°C. The membrane was then incubated for 3 h at room temperature with the appropriate antibody diluted in TBST: rabbit anti-$P2X_4$ and anti-$P2X_7$, (1/300, Alomone Laboratories) rabbit anti-GFP (1:2,000, Torrey Pines), rabbit anti-BDNF (1:100, Santa Cruz) and mouse anti-tubulin (1:2,500, Sigma). After three washes in TBST, the membrane was then incubated with species-specific HRP-conjugated secondary antibody for 1 h at room temperature and revealed with ECL+ detection kit (GE Healthcare).

YO-PRO uptake. Cells were incubated for 10 min with YO-PRO (1 μM, Invitrogen) in a divalent-free solution containing (in mM): NaCl 145, KCl 3, CaCl2 0.1, Hepes 10, pH 7.3 and stimulated with 1 mM ATP in the low divalent solution for 1 min. Fluorescence was excited at 350 nm and emitted light was collected above 540 nm. Images were acquired every 2 s. Analysis of fluorescence was performed with Metaview software. Results are expressed as the mean fluorescence expressed in arbitrary units of all recorded cells (>50) after background subtraction.

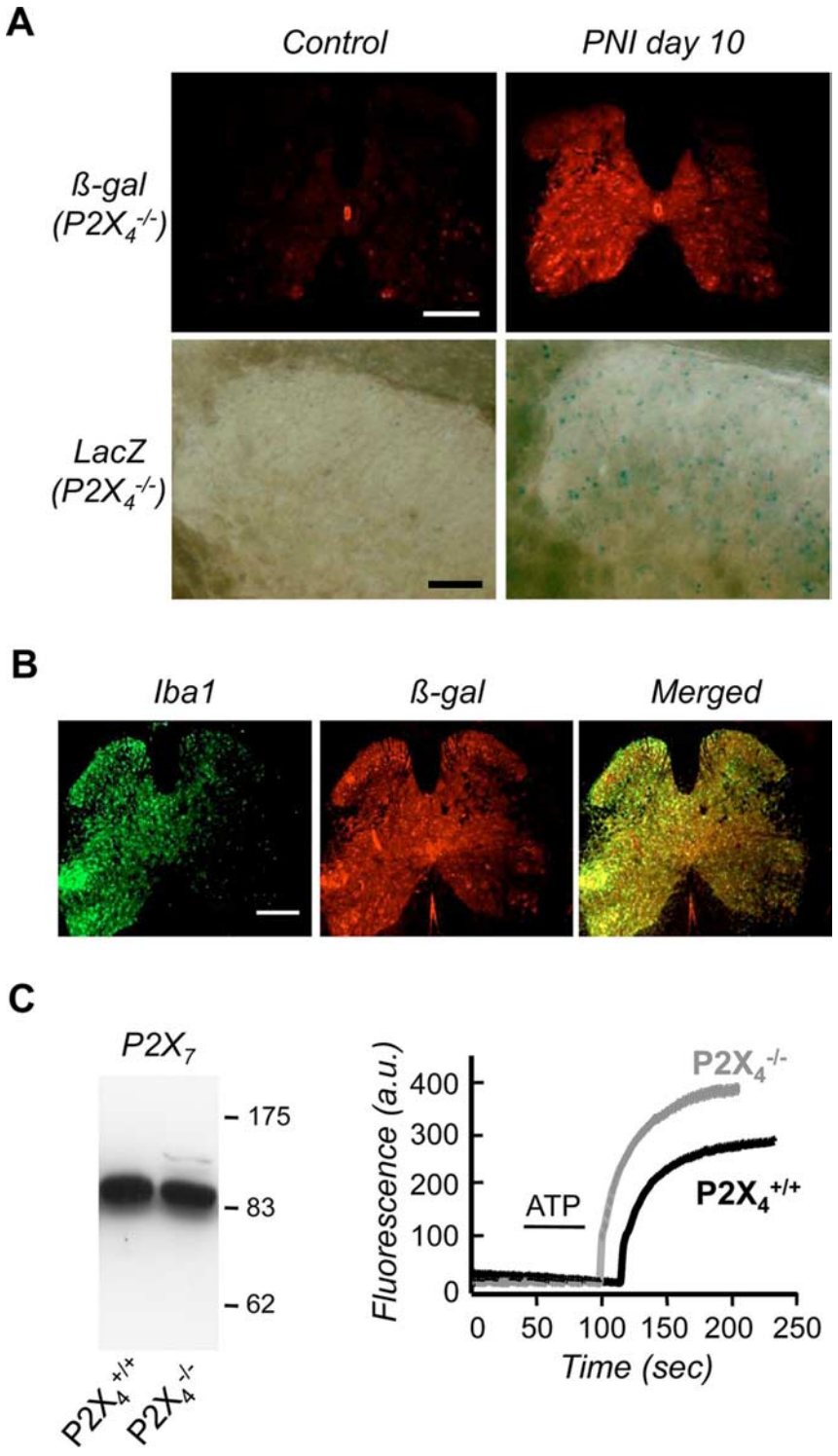

Figure 2. Microglial upregulation of *P2X4* gene expression induced by PNI is still present in $P2X_4$-deficient mice. ***A***, In $P2X_4^{-/-}$ mice, PNI induced β-galactosidase expression in the spinal cord ipsilateral to the lesion whereas no expression was detected in sham animals. Top row, β-Galactosidase immunoreactivity in spinal cord; bottom row: LacZ staining in the dorsal horn (scale bar, 500 μm). ***B***, Post-PNI, ß-galactosidase immunoreactivity (red) colocalized with the microglial marker Iba1 (green). Scale bar, 500 μm. ***C***, $P2X_4$-gene deletion did not affect $P2X_7$ expression and function in microglia. Western blot of $P2X_7$ expression (left) and ATP (1 mM) induced YOPRO uptake in primary microglial culture from wild-type and $P2X_4^{-/-}$ mice (right). Representative experiment of 3 showing mean fluorescence intensity of all recorded cells (>50) after background substraction.

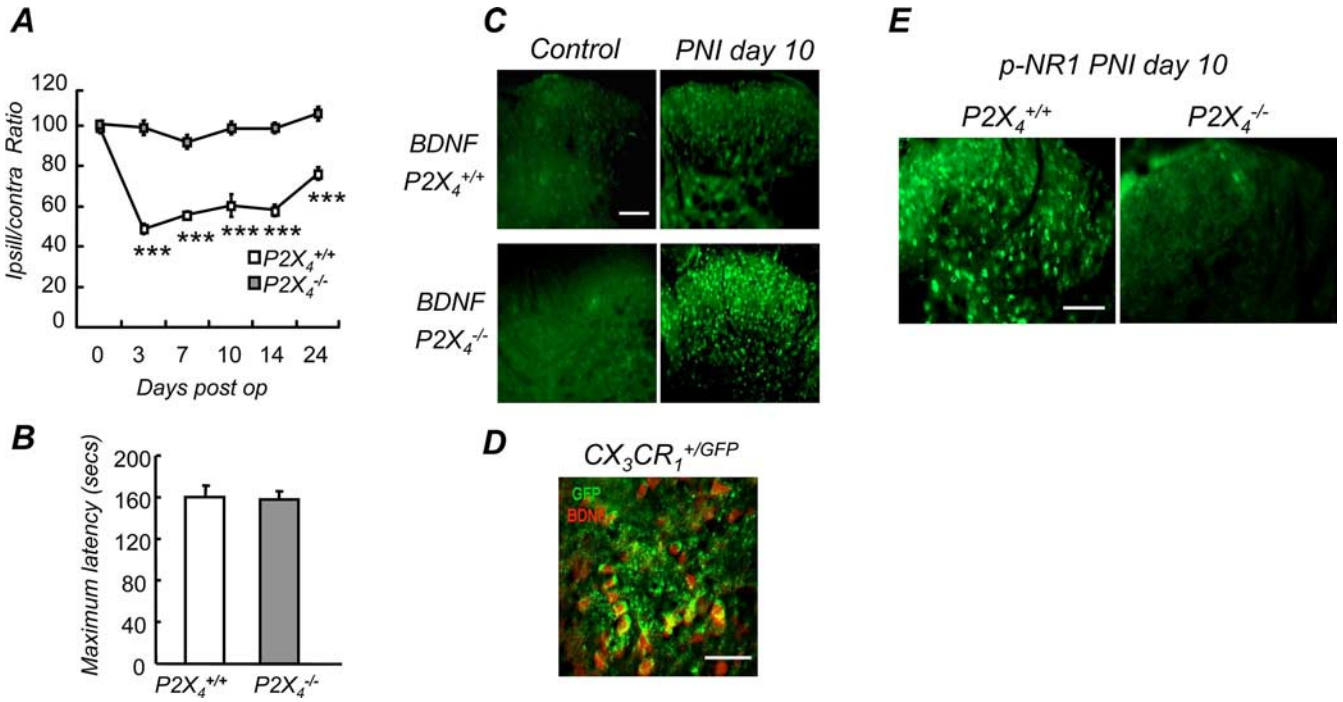

Figure 3. Lack of mechanical hypersensitivity and altered BDNF signaling in $P2X_4$-deficient mice after peripheral nerve injury. ***A***, Time course of mechanical hypersensitivity induced by sciatic nerve ligature in wild-type and $P2X_4^{-/-}$ mice expressed as ipsilateral/contralateral ratios. No hypersensitivity was observed in the $P2X_4^{-/-}$ mice, whereas wild-type mice develop a strong hypersensitivity lasting >23 d, ($n = 15$ mice, two-way ANOVA, ***$p < 0.005$). ***B***, Sensorimotor coordination was measured using the rotarod test. No difference between wild-type and $P2X_4^{-/-}$ mice was observed, ruling out a potential motor deficit in the $P2X_4^{-/-}$ mice. ***C***, BDNF immunoreactivity in the dorsal horn of the spinal cord was analyzed by immunohistochemistry in wild-type and $P2X_4^{-/-}$ mice. In sham-operated mice, BDNF immunoreactivity was not detected in either wild-type or $P2X_4^{-/-}$ animals (left). Ten days post-PNI (PNI day 10), BDNF immunoreactivity was slightly increased in wild-type mice, whereas a strong immunoreactivity was observed in the $P2X_4^{-/-}$ mice. Scale bar, 100 μm. ***D***, In the dorsal horn of $CX3CR1^{+/GFP}$ mice 10 d post-PNI, BDNF immunoreactivity (red) colocalized with microglial eGFP fluorescence (green). Scale bar, 50 μm. ***E***, PNI-induced phosphorylation of the NR1 subunit in dorsal horn neurons was impaired in $P2X_4^{-/-}$ mice. Levels of phosphorylation of NR1 were analyzed by immunohistochemistry. Scale bar, 100 μm.

Results

$P2X_4R$ expression in the spinal cord after PNI was analyzed by immunohistochemistry in WT mice. Ten days after nerve injury, $P2X_4R$ expression in the spinal cord was strongly upregulated, ipsilateral to the lesion, whereas in sham animals its presence was barely detectable (Fig. 1*A*). To investigate the relationship between microglia activation and $P2X_4R$ expression, we took advantage of the $CX3CR1^{+/GFP}$ mice, in which eGFP is specifically expressed in microglia within the CNS (Jung et al., 2000). At low magnification, images of the spinal cord showed an increase of the microglial fluorescence ipsilateral to the lesion (10 d post-PNI) compared with control (Fig. 1*B*). At higher magnification, the typical morphological modifications of activated microglia (thicker and shorter processes, larger cell body) were clearly detected in the dorsal horn, post-PNI compared with sham. In addition, a clear colocalization of eGFP and $P2X_4R$ immunostaining further confirmed that after PNI, expression of $P2X_4R$ is upregulated in activated microglia (Fig. 1*C*).

Microglial activation after PNI was analyzed in $P2X_4^{-/-}$ mice. We first investigated whether the transcriptional upregulation of the $P2X_4$ gene induced by a nerve lesion was still present in $P2X_4$-deficient mice. Ten days post-PNI a strong increase of β-galactosidase immunostaining was present in the spinal cord, ipsilateral to the lesion. LacZ staining was also observed, particularly in the dorsal horn of the spinal cord (Fig. 2*A*). As observed for $P2X_4$ immunoreactivity in $P2X_4^{+/+}$ sham-operated mice, the level of β-galactosidase expression in the $P2X_4^{-/-}$ sham-operated mice was very low. Microglial activation in $P2X_4^{-/-}$ mice was analyzed by immunostaining with Iba1 antibody, a specific marker of microglia. Post-PNI a strong Iba1 staining was found ispilateral to the lesion whereas little staining could be observed on the contralateral side (Fig. 2*B*). In addition coimmunostaining with ß-galactosidase revealed a strong colocalization of both markers. Microglial $P2X_7$ expression in the $P2X_4$-deficient mice was also monitored *in vitro*. Both Western blotting and YOPRO uptake experiments demonstrated that $P2X_7$ expression was not different between $P2X_4^{+/+}$ and $P2X_4^{-/-}$ mice (Fig. 2*C*). Together, these results demonstrate that $P2X_4$ gene deletion does not affect microglial activation *in vivo* and *in vitro*.

Mechanical hypersensitivity induced by PNI was measured using a Randall-Selitto test in both $P2X_4^{+/+}$ and $P2X_4^{-/-}$ mice. $P2X_4^{+/+}$ mice developed strong mechanical hyperalgesia throughout the testing period (days 3–24) ($p < 0.005$), whereas in the $P2X_4^{-/-}$ mice no significant difference was observed compared with baseline (Fig. 3*A*). Analysis of rotarod data did not reveal any significant difference in latencies between $P2X_4^{-/-}$ and wild-type mice (Fig. 3*B*), ruling out a possible implication of $P2X_4R$ expressed by spinal motor neurons in the observed phenotype.

ATP-mediated BDNF release from microglia has been linked to the development of allodynia associated with nerve injury (Coull et al., 2005). We investigated whether BDNF signaling in the spinal cord was altered in $P2X_4^{-/-}$ mice after peripheral nerve lesion. Ten days postinjury, BDNF immunostaining in the dorsal horn was strongly enhanced in $P2X_4^{-/-}$ compared with wild-type mice; no difference between genotypes was observed in sham-operated animals (Fig. 3*C*). After PNI, BDNF immunostaining colocalized with the microglial marker GFP in the $CX3CR1^{+/GFP}$ mice (Fig. 3*D*). BDNF is known to induce phosphorylation of the NR1 subunit of

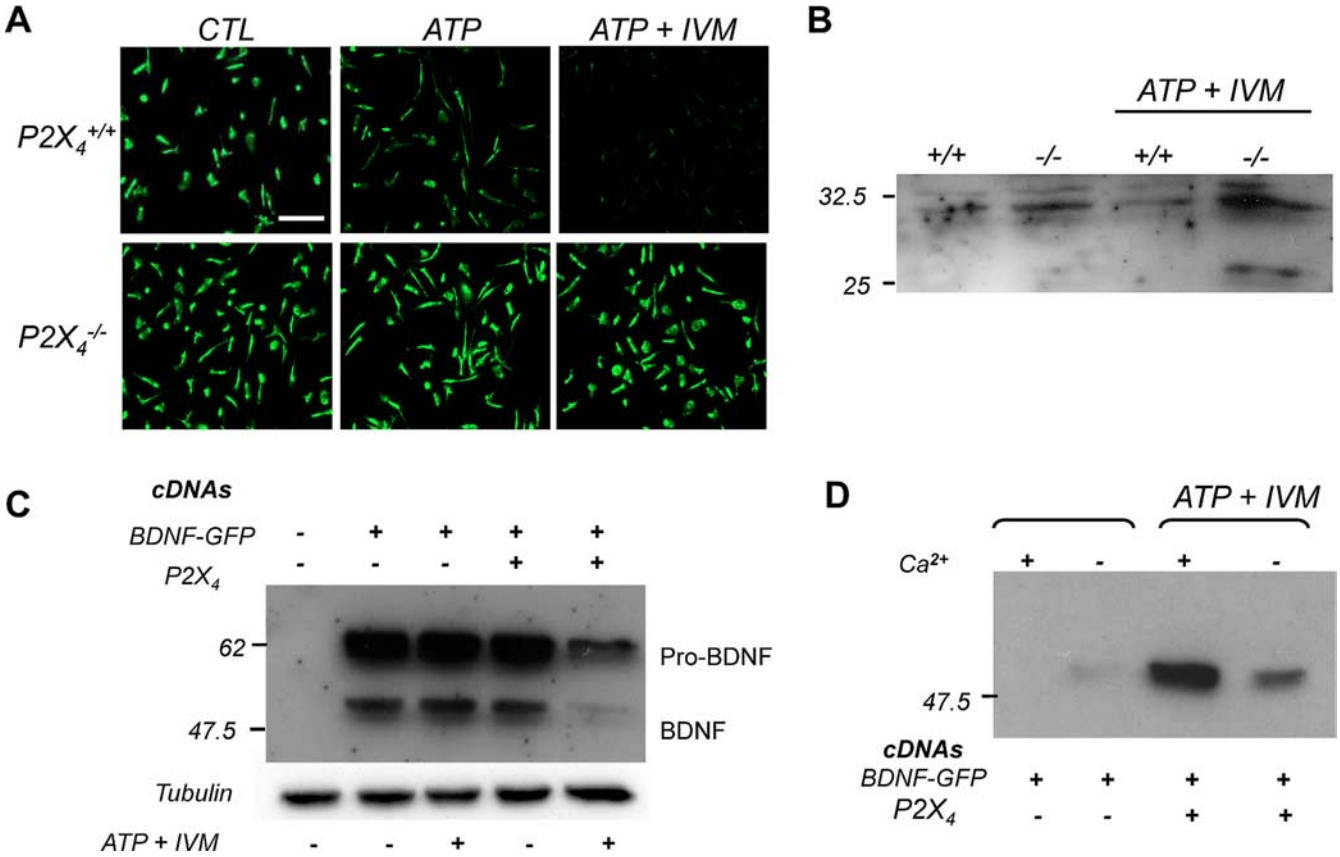

Figure 4. $P2X_4$ stimulation triggers BDNF release from activated microglia. ***A***, ATP-evoked release of BDNF from wild-type and $P2X_4^{-/-}$ microglia primary cultures. Intracellular BDNF content of microglia was analyzed by immunostaining. Stimulation of a culture with ATP (100 μM) alone or coapplied with ivermectin (IVM, 3 μM) induced a strong decrease of BDNF immunoreactivity in a primary microglial culture from wild-type mice. In a culture from $P2X_4^{-/-}$ mice, ATP or ATP+IVM stimulation did not induce any detectable changes in BDNF immunoreactivity. The data shown are representative of six experiments. Scale bar, 100 μm. ***B***, Western blot of intracellular BDNF content from primary cultures of wild-type and $P2X_4^{-/-}$ microglia. Cells were stimulated as above. The top band corresponds to pro-BDNF; the mature form (i.e., the 14 kDa form) could not be resolved in these experiments. ATP+IVM stimulation induced a reduction of intracellular pro-BDNF in wild-type but not in $P2X_4^{-/-}$ cultures. The intermediate form seen in stimulated $P2X_4^{-/-}$ cultures is of unknown origin. ***C***, ***D***, $P2X_4$ activation triggered BDNF release in a recombinant expression system. $P2X_4$ and BDNF-GFP cDNAs were transiently transfected in COS-7 cells either alone or in combination. Forty-eight hours later, cells were stimulated or not with ATP+IVM and intracellular (***C***) or secreted (***D***) BDNF-GFP was analyzed by Western blotting. Note that in the absence of extracellular Ca^{2+}, ATP-induced secretion of BDNF was strongly reduced.

the NMDA receptors expressed in dorsal horn neurons (Slack et al., 2004). Ten days post-PNI, immunofluorescence of p-NR1 was strongly reduced in $P2X_4^{-/-}$ mice when compared with wild-type animals (Fig. 3*E*). Altogether these results suggest that BDNF released from microglia is impaired in $P2X_4^{-/-}$ mice.

ATP-induced secretion of BDNF by microglia was further investigated in microglia primary cultures from wild-type and $P2X_4^{-/-}$ mice. Intracellular BDNF content was analyzed by immunostaining. In wild-type cultures, stimulation with 100 μM ATP induced a reduction of intracellular BDNF staining that was more pronounced in the presence of 3 μM ivermectin (IVM), a positive allosteric modulator of $P2X_4R$ (Fig. 4*A*). In contrast, in cultures from $P2X_4^{-/-}$ mice, neither ATP nor ATP+IVM stimulations produced an alteration of intracellular BDNF content. These results were obtained using two different commercial anti-BDNF antibodies (data not shown). Corroborating observations were made when BDNF cellular content was analyzed by Western blotting (Fig. 4*B*). $P2X_4R$-mediated BDNF release was also tested in a recombinant system. COS-7 cells were transfected with $P2X_4R$ and BDNF-GFP cDNAs either alone or in combination. Cells were stimulated by ATP+IVM and both cellular and secreted BDNF-GFP content analyzed by Western blotting. When $P2X_4$ and BDNF-GFP were coexpressed, ATP+IVM induced a marked decrease of intracellular BDNF content (Fig. 4*C*); conversely, secretion of BDNF-GFP in the medium was increased (Fig. 4*D*). In addition, omitting calcium from the extracellular medium abolished ATP-mediated BDNF release.

Discussion

Purinergic signaling appears to be a key pathway regulating microglial response to injury and nerve degeneration (Färber and Kettenmann, 2006). In this study using we show that in wild-type mice, PNI induces a strong upregulation of $P2X_4R$ expression in the spinal cord ispsilateral to the lesion, whereas it is barely detectable in sham animals. In line with previous studies (for review, see Inoue, 2006; Scholz and Woolf, 2007), our experiments performed in the $CX3CR1^{+/GFP}$ mice confirm that peripheral nerve injury induces activation of spinal microglia that is characterized by typical morphological changes. In addition, our results provide clear evidence that the induction of $P2X_4$ expression in the spinal cord resulting from peripheral nerve lesion is restricted to activated microglia.

ATP released from damaged cells has been proposed to be a triggering factor of microglial activation (Inoue, 2002). However, in $P2X_4$-deficient mice microglial activation subsequent to PNI is not affected. Indeed, morphological changes and $P2X_7$ expression, both of which are associated with microglial activation, were unaffected in $P2X_4^{-/-}$ mice. Interestingly, PNI-mediated transcriptional upregulation of $P2X_4$ gene was still observed in KO mice. These observa-

tions therefore rule out a possible involvement of $P2X_4R$ in ATP-mediated microglial activation. Rather they suggest that within the CNS, $P2X_4$ expression could represent a physiological marker of microglial activation.

$P2X_4R$ deletion results in a complete absence of mechanical hypersensitivity subsequent to peripheral nerve lesion, while leaving motor coordination untouched. We also provide evidence that tactile allodynia is reduced in $P2X_4^{-/-}$ mice after spared nerve injury, a different model of neuropathic pain (supplemental Fig. 1, available at www.jneurosci.org as supplemental material). These behavioral phenotypes are in agreement with a previous study using intrathecal injections of $P2X_4R$ antisense oligonucleotides into rat spinal cord before PNI (Tsuda et al., 2003) and further demonstrate the direct involvement of microglial $P2X_4R$ in the establishment of mechanical hypersensitivity associated with neuropathic pain.

After PNI, ATP-mediated BDNF release from activated microglia likely mediates the downregulation of the K^+/Cl^- cotransporter KCC2 in inhibitory interneurons, which in turn gates allodynia (Coull et al., 2003, 2005). Our results provide a direct demonstration that $P2X_4R$ controls the release of BDNF from activated microglia subsequent to peripheral nerve damage. This conclusion is provided by our findings that in $P2X_4$-deficient mice, nerve injury results in BDNF accumulation in dorsal horn microglia whereas phosphorylation of the NR1 subunit of the NMDA receptor is strongly decreased. This reduction of p-NR1 levels in dorsal horn neurons of $P2X_4R$ deficient mice may also account for the lack of hypersensitivity after PNI, suggesting that $P2X_4R$-induced BDNF release from microglia not only promotes allodynia but may also contribute to longer term enhancement of synaptic strength (Kerr et al., 1999). Finally, using primary microglia cultures and a recombinant expression system, our results clearly demonstrate the direct involvement of $P2X_4R$ in ATP-mediated BDNF.

This work and recent studies highlight the roles of purinergic P2 receptors as critical regulators of microglial functions. In homeostatic brain, metabotropic $P2Y_{12}$ receptors regulates microglial branch dynamics (Davalos et al., 2005), whereas in activated states $P2X_4$, $P2X_7$, $P2Y_{12}$ or $P2Y_6$ receptors are involved in the secretion of proinflammatory mediators, chemotaxis or in phagocytosis (Kettenmann, 2007). These studies also support the idea that purinergic signaling is central to bidirectional communications between microglia and other brain cells. Indeed, microglia, in either resting or activated states, can sense ATP released by local network activity through an array of purinergic receptors, which expression profile depends on activation states. There is now a growing number of evidence suggesting that activation of these receptors, particularly $P2X_4$ and $P2X_7$, can promote neuronal excitability. This is of particular importance in chronic brain disease at which sustained activation of these microglial receptors can promote long term modifications of synaptic strength or excitotoxic damages (Färber and Kettenmann, 2006). Targeting these receptors may well represent an attractive therapeutic alternative to NSAID treatment of chronic pain syndromes and/or to limit some deleterious effect of inflammation associated with neurodegenerative diseases.

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