Expression and Function of Lysophosphatidic Acid Receptors in Cultured Rodent Microglial Cells

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Thomas Möller‡§, James J. Contos¶, David B. Musante‡, Jerold Chun‡, and Bruce R. Ransom‡

From the Departments of ‡Neurology and ¶Neurological Surgery, School of Medicine, University of Washington, Seattle, Washington 98195 and the §Department of Pharmacology, School of Medicine, University of California, San Diego, California 92039

Microglia are the resident tissue macrophages of the central nervous system. They are rapidly activated by a variety of insults; and recently, receptors linked to cytoplasmic Ca\(^{2+}\) signals have been implicated in such events. One potential class of receptors are those recognizing lysophosphatidic acid (LPA). LPA is a phospholipid signaling molecule that has been shown to cause multiple cellular responses, including increases in cytoplasmic calcium. We examined whether any of the known LPA receptor genes (lpA1, lpA2, and lpA3) are expressed by cultured mouse or rat microglia. Reverse transcriptase-polymerase chain reaction indicated that mouse microglia predominantly expressed the lpA1 gene, whereas rat microglia predominantly expressed lpA3. Although LPA induced increases in the cytoplasmic Ca\(^{2+}\) concentration in both microglial preparations, the responses differed substantially. The Ca\(^{2+}\) signal in rat microglia occurred primarily through Ca\(^{2+}\) influx via the plasma membrane, whereas the Ca\(^{2+}\) signal in mouse microglia was due to release from intracellular stores. Only at high concentrations was an additional influx component recruited. Additionally, LPA induced increased metabolic activity in mouse (but not rat) microglial cells. Our findings provide evidence for functional LPA receptors on microglia. Thus, LPA might play an important role as a mediator of microglial activation in response to central nervous system injury.

Microglia are the resident immune cells of the central nervous system (1). Once activated during a central nervous system insult, microglial cells undergo a rapid, graded response that involves cell migration, proliferation, cytokine release, and trophic and/or cytotoxic effects (1, 2). The signals and mechanisms of microglial activation following central nervous system injury are just beginning to be identified. Although several cytokines, growth factors, and peptides are known to stimulate microglia via receptors on the cell surface (for review, see Ref. 3), other molecules are likely to have important roles as well.

One signaling molecule that might regulate microglial activation during central nervous system injury is lysophosphatidic acid (LPA). LPA is a bioactive phospholipid mediator that is produced by activated platelets, injured cells, and cells stimulated by growth factors (4–7). LPA was initially shown to cause multiple biological effects on fibroblasts, including increases in the cytoplasmic Ca\(^{2+}\) concentration ([Ca\(^{2+}\)\(_{i}\)]) and increased proliferative activity (8, 9). The effects of LPA are mediated through three distinct G-protein-coupled receptors that are encoded by different genes (lpA1/Edg2, lpA2/Edg4, and lpA3/Edg7) (for review, see Refs. 5–7). The three LPA receptors differ somewhat in their signal transduction properties. Although activation of any of the three receptors results in increased [Ca\(^{2+}\)\(_{i}\)]\(_{o}\), only the LPA1-mediated response is completely blocked by pertussis toxin (PTX) (10, 11). All three receptors mediate inhibition of adenylate cyclase, although LPA1 is most effective (7, 12). In addition, although LPA1 and LPA2 receptors mediate acute neurite retraction, the LPA3 receptor does not (7, 12).

Recent data suggest that LPA may play a prominent role in the central nervous system. Astrocytes are known to respond to LPA with [Ca\(^{2+}\)\(_{i}\)]\(_{o}\) increases, lipid peroxidation, DNA synthesis, and cell rounding (13–16). Oligodendrocytes respond to LPA with [Ca\(^{2+}\)\(_{i}\)]\(_{o}\) increases (17) and have been shown to express the lpA1 gene in vivo (18). Neuroblasts, which also express the lpA1 gene, respond to LPA with cell rounding, proliferation, increased chloride conductances, and activation of a nonselective cation conductance (19, 20). Mature neurons respond to LPA with [Ca\(^{2+}\)\(_{i}\)]\(_{o}\) increases, rapid growth cone collapse, and neurite retraction (21, 22). LPA has also been shown to increase the tight junction permeability of cerebral endothelium and to alter cerebrovascular reactivity (23, 24). To date, however, the effects of LPA on microglial cells have not been investigated.

In this study, we show that cultured mouse and rat microglia each express LPA receptor genes, albeit different ones. We show that the receptors possess functional signal transduction properties, as the cells respond to LPA with [Ca\(^{2+}\)\(_{i}\)]\(_{o}\) signals and increased metabolic activity. Although specific signal transduction aspects may differ between species, our results suggest that LPA is an important signaling molecule that might activate microglial cells in response to central nervous system injury.

EXPERIMENTAL PROCEDURES

Preparation of Mouse and Rat Microglial Cells—Microglial cells were prepared from the cortices of newborn (postnatal days 1–3) Swiss-Webster mice or Long-Evans rats as described previously (25, 26). In brief, cortical tissue was freed from blood vessels and meninges. Tissue was minced, trypsinized for 20 min, triturated with a fire-polished
pipette, and washed twice. The cortical cells were cultured in Dulbecco’s modified Eagle’s medium/nutrient mixture F-12 supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin (all from Life Technologies, Inc.) and 20% L929 conditioned medium. Cells were kept at 37 °C in a 5% CO2 and 95% air incubator, and the fluorescence emission was collected at a wavelength of 510 ± 20 nm via a band-pass filter. After 9 days, the microglial cells were separated from the underlying astrocytic monolayer by gentle agitation using their differential adhesive properties. The resulting cell suspension was spun down at 200 × g for 10 min. The cell pellet was resuspended in Dulbecco’s modified Eagle’s medium/nutrient mixture F-12 with 10% fetal bovine serum. For imaging experiments, microglial cells were plated on poly-l-lysine-coated glass coverslips as mixture was cycled 35 times at 95 °C for 30 s, 56 °C for 30 s, and 72 °C for 30 s. Each PCR condition was overlaid with mineral oil and heated to 90 °C). Reaction mixtures were cycled 35 times at 95 °C for 5 min; cooled on ice; diluted to 100 μl each dNTP, 0.5 mM each primer, 9.0) and 15 mM (NH4)2SO4), 0.25 mM each dNTP, 0.5 mM each dNTP, 0.5 mg/ml RNasin (Roche Molecular Biochemicals), and 0.5 units of Superscript (Life Technologies, Inc.). The reaction mixture was cycled 35 times at 95 °C for 5 min, and 95 °C for 5 min; cooled on ice; diluted to 100 μl with H2O and stored at −20 °C. The PCR products were confirmed by agarose gel electrophoresis and purified using the Wizard PCR Prep Kit (Promega, Madison, WI) and the QIAquick PCR Purification Kit (Qiagen, Valencia, CA). The DNA purity and concentration of each PCR product was determined using a spectrophotometer.

**RESULTS**

**LPA Receptor Gene Expression in Cultured Microglial Cells**—To generate a cDNA template, total RNA from cultured microglial cells was isolated using the RNeasy minikit (QIAGEN Inc., Valencia, CA) according to the manufacturer’s instructions. For reverse transcription, a 40-μl reaction mixture consisting of 1× Superscript first-strand synthesis buffer, 20 units of RNAsin (Roche Molecular Biochemicals), and 200 units of Superscript (Life Technologies, Inc.) was incubated at 23 °C for 10 min, 42 °C for 60 min, and 95 °C for 5 min; cooled on ice; diluted to 100 μl with H2O, and stored at −20 °C. The PCR products were confirmed by agarose gel electrophoresis and purified using the Wizard PCR Prep Kit (Promega, Madison, WI) and the QIAquick PCR Purification Kit (Qiagen, Valencia, CA). The DNA purity and concentration of each PCR product was determined using a spectrophotometer.

**Assay for Microglial Metabolic Activity/Proliferation**—Microglial metabolic activity was assessed in 96-well plates with 2 × 105 cells/well in 150 μl of RPMI 1640 medium (Life Technologies, Inc.). LPA (1, 3, 10, and 30 μM) was directly dissolved in RPMI 1640 medium supplemented with lipid-free bovine serum albumin (0.1%) to improve the solubility of LPA. Controls received the carrier only. 1–2 days after plating, cells were stimulated with LPA for 1 h, carefully washed with Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum. For assessing the metabolic activity 24 and 48 h later, cells were incubated with 15 μl of the cell proliferation reagent WST-1 (Roche Molecular Biochemicals) for 30–60 min. This colorimetric assay is based on the cleavage of the tetrazolium salt WST-1 by mitochondrial dehydrogenase in viable cells. The amount of cleaved WST-1 is proportional to the metabolic activity of mitochondria and can be used as a readout for metabolic activity and/or proliferation. The assay was used in accordance with the manufacturer’s instructions. Absorption was measured at 450 nm with 750 nm as reference in a microplate reader (EL 340, Bio-Tek Instruments, Winooski, VT). Metabolic activity/proliferation is given as percent change compared with control. All experiments were carried out with a minimum of six wells/condition (n ≥ 6).

**Solutions and Reagents**—Standard HEPES bathing solution for experiments with cultured cells was composed of 150 mM NaCl, 2 mM KCl, 2 mM CaCl2, 1 mM MgCl2, 10 mM HEPES/NaOH, 10 mM glucose (pH 7.35). To obtain Ca2+-free solution, CaCl2 was omitted; MgCl2 was increased to 2 mM, and 0.5 mM EGTA was added. For the application of lidocaine (40 μM), NaCl was reduced to 110 mM. Reagents were prepared from stock solutions stored at −20 °C by dilution in standard or calcium-free extracellular bathing solution immediately prior to use. If not stated otherwise, chemicals were obtained from Sigma.

**Statistics**—Statistical evaluation was carried out using Prism software (GraphPAD, San Diego, CA). Comparisons were made by analysis of variance with Bonferroni’s post-test, and p < 0.05 was considered to be significant. Data are given as means ± S.E.

**RESULTS**

**LPA Receptor Gene Expression in Cultured Microglial Cells**—To examine expression of the three known LPA receptor genes (lpA1/Edg2, lpA2/Edg4, and lpA3/Edg7) in microglial cells, we utilized RT-PCR (Fig. 1). Primers were designed to sequence conserved between mouse and human sequences. Robust cDNA amplification from rat testes and kidney, two tissues that express all three receptor genes, showed that the primers also recognized rat sequence. In three independent primary microglial cell cultures from mouse, we found that lpA2 was the primary LPA receptor gene expressed, with little or no lpA3 and lpA2. In contrast, three independent primary microglial cell cultures from rat expressed primarily lpA3, with little or no lpA3 and lpA2. We also examined expression of the three receptors in two microglial cell lines. The mouse N9 cell line
expressed only lPA2, whereas BV2 expressed none of the receptors. These results suggest that microglial cells from mouse and rat would respond to LPA, although these responses might differ based on the distinct coupling properties of the three receptors.

LPA-induced [Ca\textsuperscript{2+}] Signals in Rat Microglial Cells—To investigate the ability of LPA to induce [Ca\textsuperscript{2+}] signals in cultured microglia, Fura-2-loaded cells were stimulated with varying concentrations of LPA in a perfusion chamber. We initially determined that individual rat microglial cells had variable responses to the same concentrations of LPA, although increasing LPA concentrations resulted in a higher probability of response (Fig. 2A, insets). This variability at the level of single cells was probably due to the poor solubility of LPA in aqueous solutions and the resulting formation of micelles. To compensate for this, average population responses were determined, which facilitated quantitative comparisons. Microglial cells were stimulated consecutively with 0.03, 0.1, 0.3, 1.0, and 3 \mu M LPA. In the shown experiment (Fig. 2A), the percentage of rat microglial cells responding with a [Ca\textsuperscript{2+}], signal (defined as \( \geq 1.2 \times \text{baseline} \)) to LPA stimulation increased in a concentration-dependent fashion from 15% for 30 \text{nM} LPA to 100% for 3 \mu M LPA (Fig. 2A). No response was seen at 10 \text{nM} in any experiment. Pooled data from several experiments were used to construct a concentration-response graph (Fig. 2B). Repeated application of LPA (e.g., three applications of \( \geq 3 \mu M \) LPA for 30 s, with 5-min intervals) did not lead to a reduction in the number of cells responding or a significant decrease in the response amplitude (data not shown). Based on these findings, we used 3 \mu M LPA for our further studies. We did not use higher LPA concentrations, as they would have necessitated increased solvent (Me\textsubscript{2}SO) concentrations of \( >0.3\% \).

LPA-induced [Ca\textsuperscript{2+}] Signals in Mouse Microglial Cells—Mouse microglial cells showed a similar heterogeneity in responses of individual cells (data not shown). Nevertheless, LPA induced [Ca\textsuperscript{2+}], signals with two different kinetic profiles. At concentrations \( \leq 1 \mu M \), LPA induced a transient [Ca\textsuperscript{2+}], signal. At higher concentrations, LPA induced a [Ca\textsuperscript{2+}], signal with an initial peak-like component, followed by a long-lasting [Ca\textsuperscript{2+}], elevation, which depended partially on extracellular Ca\textsuperscript{2+} (Fig. 3A). In contrast to rat microglia, the LPA-induced [Ca\textsuperscript{2+}], signals in mouse microglia showed a pronounced desensitization to repeated application of LPA (Fig. 3, B and C). At concentrations \( \geq 1 \mu M \), LPA, the number of cells responding to a second or third application was reduced by \( \sim 50\% \); but between LPA applications, the [Ca\textsuperscript{2+}], returned to the base line. At 3 \mu M LPA, the number of cells responding to repeated LPA application was reduced by only \( \sim 30\% \); but after the first LPA application, [Ca\textsuperscript{2+}], was maintained at a new steady-state level above the base line, and the transients had a substantially reduced amplitude. Because of this desensitization, the concentration-response graph shown in Fig. 2B was obtained from initial responses in separate experiments. In contrast to the rat cells, which showed responses only at concentrations \( \geq 30 \text{nM} \), mouse microglial cells showed responses to LPA concentrations as low as 3 \text{nM}.

Source of the [Ca\textsuperscript{2+}], Increases in Rat Microglial Cells—LPA-induced increases in [Ca\textsuperscript{2+}], have been shown to be due both to influx through the plasma membrane and to inositol 1,4,5-trisphosphate-mediated release from intracellular stores (10,
ATPase and results in a depletion of endoplasmic Ca$^{2+}$ stores. First, we observed a normal LPA-induced [Ca$^{2+}$]$_i$ transient. This hypothesis was further supported by three experiments. The sodium channel blocker known to interfere with LPA-agonist [Ca$^{2+}$]$_i$ transient was completely abolished by La$^{3+}$, a compound that inhibits the endoplasmic Ca$^{2+}$ stores, whereas at high LPA concentrations, an additional plasma membrane influx pathway is recruited. This transmembrane influx was blocked by Ca$^{2+}$-free buffer, lidocaine (40 mM) (data not shown), or La$^{3+}$ (10 mM) (Fig. 5C).

G-proteins Mediating the [Ca$^{2+}$]$_i$ Signal—To investigate which G-proteins mediate the [Ca$^{2+}$]$_i$ signal in microglia, cells were preincubated with 1 μg/ml PTX for 2 h. This treatment, which ADP-ribosylates and inhibits G$_i$/G$_o$ proteins, was shown to be effective in cultured microglial cells (26). PTX did not prevent LPA-induced [Ca$^{2+}$]$_i$ transients in rat microglial cells (Fig. 5E), but completely blocked the response in mouse microglial cells (Fig. 5D).

LPA-induced Increases in Microglial Metabolic Activity/Proliferation—Increased metabolic activity and/or proliferation is a common feature of activated microglial cells. To investigate if LPA induces increases in microglial metabolic activity/proliferation, cells were incubated with LPA (1, 3, 10, or 30 μM) for 1 h. Metabolic activity/proliferation was assessed 24 and 48 h later using the WST-1 assay, which relies on the measurement of mitochondrial activity as a readout for metabolic activity and/or proliferation. We found that LPA induced concentration-dependent increases in metabolic activity in mouse microglial cells after 24 and 48 h, whereas rat microglial cells did not show such increases (Fig. 6).

FIG. 3. LPA-induced [Ca$^{2+}$]$_i$ signals in cultured mouse microglial cells. A. Fura-2-loaded mouse microglial cells were perfused with varying concentrations of LPA. LPA concentrations ≤1 μM induced a transient [Ca$^{2+}$]$_i$ signal, whereas 3 μM LPA induced a long-lasting [Ca$^{2+}$]$_i$ signal, partially dependent on extracellular Ca$^{2+}$ stores, whereas at high LPA concentrations, an additional plasma membrane influx pathway is recruited. This transmembrane influx was blocked by Ca$^{2+}$-free buffer, lidocaine (40 mM) (data not shown), or La$^{3+}$ (10 mM) (Fig. 5C).

DISCUSSION

Molecular Receptor Characterization—The effects of LPA are mediated through at least three known G-protein-coupled receptors encoded by different genes ($lpA_1$/Edg2, $lpA_2$/Edg4, and $lpA_3$/Edg7) (for review, see Refs. 5 and 6). Although we showed that LPA receptor genes are expressed by cultured microglial cells from mouse and rat, it was surprising to find that different receptor subtypes were expressed by the different species. Primary rat microglial cultures predominantly expressed $lpA_3$, whereas primary mouse microglial cultures predominantly expressed $lpA_1$. The different receptor genes expressed by the different species suggested that different LPA responses might be observed in the different cultures since the receptor subtypes differ somewhat in their coupling specificities (7, 11, 12).

It has previously been shown by Northern blotting that $lpA_1$ is expressed in the developing and adult mouse central nervous system (18) and in the adult human brain (34). Although $lpA_1$ was shown to co-localize with proteolipid protein in adult brain (indicating expression in oligodendrocytes), double staining was not performed with microglial specific markers (18). Generalized adult brain expression of both $lpA_2$ and $lpA_3$ is low in both murine and human species (34–36). Nevertheless, the relatively small percentage of microglial cells in regard to the total brain volume (37) may lead to a very low signal on a total brain RNA blot. Thus, it remains possible that these genes are expressed by microglia in adult brain, but Northern blotting is not sensitive enough to pick up the signal. Additionally, the $lpA$ genes might be up-regulated due to injury-type signals caused...
by the culturing process (e.g. cell dissociation), indicating the expression in microglial cells of a higher activation state.

In the examined microglial cell lines, we found that mouse N9 and BV2 cells had receptor profiles different from those of the primary cells. This might be attributed to cellular changes due to immortalization and suggests caution in extrapolating observations from cell lines to primary cells.

It seems important to keep in mind that the main body of data available on LPA receptor expression (including this study) shows expression at the mRNA level. As the amount of actual protein expressed does not necessarily match the amount of mRNA, one has to be cautious with the interpretation of the data. Nevertheless, the observed differences in cellular responses might indicate an actual difference in functional LPA receptor expression. Further scrutinizing of LPA receptor expression awaits the availability of cross-species receptor antibodies.

To our knowledge, this is the first report showing species-specific expression of LPA receptor genes in a given cell type. However, LPA responses have previously been shown to differ between different species. For example, LPA induces platelet aggregation in humans, but not in rats, and leads to opposite effects on blood pressure upon intravenous injection (38, 39). As the three cloned LPA receptors differ somewhat in their signal transduction properties (7, 12), we tested whether LPA stimulation of microglial cells of different origin (and therefore different LPA receptor expression) led to different responses.

LPA-induced \([\text{Ca}^{2+}]_c\) Signals—LPA induced \([\text{Ca}^{2+}]_c\) signals in primary cultured mouse as well as rat microglial cells. In rat microglia, LPA induced \([\text{Ca}^{2+}]_c\) signals at concentrations as low as 30 nM, eliciting maximal population responses at concentrations of 3 \(\mu M\). The concentration of LPA required to elicit maximal responses in single cell varied, however. This discrepancy was most likely due to inconsistent solubility of LPA in

FIG. 4. Pharmacological modulation of the LPA-induced \([\text{Ca}^{2+}]_c\) signal in rat microglial cells. A, application of LPA (3 \(\mu M\)) in \(\text{Ca}^{2+}\)-free buffer abolished the \(\text{[Ca}^{2+}]_c\) signal (\(n = 135\)). B, preincubation with thapsigargin (500 nM, 30 min) eliminated metabotropic responses (UTP), but did not abolish the LPA (3 \(\mu M\))-induced \(\text{[Ca}^{2+}]_c\) signal, suggesting the involvement of transmembrane \(\text{Ca}^{2+}\) influx (\(n = 157\)). ATP (100 \(\mu M\)) served as a viability control. C, LPA (3 \(\mu M\)) application also failed to elicit a \(\text{[Ca}^{2+}]_c\) signal in the presence of La(3\(^{3+}\)) (100 \(\mu M\)). Application of ATP (100 \(\mu M\)) served as a control for the viability of the cells (\(n = 120\)). D, lidocaine (40 mM), a sodium channel blocker, reversibly reduced the percentage of responding cells (17% versus 98/92%, \(n = 80\)). E, preincubation with PTX (1 \(\mu g/ml, 2\) h) did not prevent the LPA-induced \(\text{[Ca}^{2+}]_c\) signal (\(n = 92\)).

FIG. 5. Pharmacological modulation of the LPA-induced \([\text{Ca}^{2+}]_c\) signal in mouse microglial cells. A, the LPA-induced \(\text{[Ca}^{2+}]_c\) signal persisted in \(\text{Ca}^{2+}\)-free medium (3 \(\mu M\)) (\(n = 172\)). B, preincubation with thapsigargin (500 nM, 30 min) blocked LPA-induced responses at 1 \(\mu M\) LPA, but 3 \(\mu M\) LPA induced a \(\text{[Ca}^{2+}]_c\) signal, which was dependent on external \(\text{Ca}^{2+}\), suggesting the involvement of transmembrane \(\text{Ca}^{2+}\) influx (\(n = 65\)). C, LPA (3 \(\mu M\)) application also failed to elicit a \(\text{[Ca}^{2+}]_c\) signal in the presence of La(3\(^{3+}\)) (10 \(\mu M\)) (\(n = 46\)). D, preincubation with PTX (1 \(\mu g/ml, 2\) h) blocked the LPA-induced \(\text{[Ca}^{2+}]_c\) signal (\(n = 100\)). The subsequent application of ATP (100 \(\mu M\)) served as a control for the viability of cells.
Other differences in the LPA-induced Ca\(^{2+}\) signals between mouse and rat cells were the higher sensitivity of mouse cells, desensitization (which was found only in mouse microglial cells), and the sensitivity to PTX (which also was seen only in mouse cells). This sensitivity to PTX is in accordance with data on heterologous expression of the receptors showing that \(I_{\text{PA}_1}\), the predominant receptor in mouse microglia, is PTX-sensitive, whereas \(I_{\text{PA}_3}\), the main receptor in rat microglial cells, is PTX-insensitive (10, 11).

**LPA-induced Increase in Microglial Metabolic Activity/Proliferation**—Increased metabolic activity/proliferation after LPA stimulation is a common feature in a wide variety of cells. Believed to account for much of the proliferative activity of serum, LPA induces proliferation in hepatocytes (42), B lymphoblasts (43), astrocytes (13), and endothelial cells (44). Interestingly, mouse microglial cells showed a concentration-dependent LPA-induced increase in microglial metabolic activity/proliferation, whereas rat microglia did not change their metabolic activity after LPA stimulation. As rat microglial cells also showed a lower LPA sensitivity in the Ca\(^{2+}\) imaging experiments, it might be that the concentrations used were not high enough to stimulate increases in metabolic activity/proliferation in rat cells. Unfortunately, higher concentrations were prevented by the limited solubility of LPA.

Interestingly, the LPA-induced increases in metabolic activity/proliferation coincided with the recruitment of the extracellular calcium influx pathway in mouse microglial cells. Nevertheless, whether this or any other signal transduction pathway is involved in the increase in microglial metabolic activity/proliferation needs to be determined in future experiments. It seems likely that the observed species-specific differences in metabolic behavior were due to the different receptors expressed. Nevertheless, the receptor subtypes that mediate changes in metabolic activity/proliferation have not been determined yet. Our results suggest that, in microglial cells, \(I_{\text{PA}_1}\) mediates changes in metabolic activity/proliferation increases, whereas \(I_{\text{PA}_3}\) does not.

**Physiological Implications of Microglial LPA Receptors**—LPA-induced morphological changes such as neurite/process retraction and proliferation have been implicated in development and in post-traumatic brain injury (6, 45). It has been postulated that LPA might be a key factor in mediating reactive gliosis and glial scar formation (6). The functional expression of LPA receptors not only in astrocytes, but also in microglial cells, indicates that microglia might be intimately involved in such pathophysiological events. Even though there is variability in the responses of different species, all primary microglia expressed LPA receptors, and both mouse and rat microglia responded to LPA stimulation with [Ca\(^{2+}\)]e signals. Future experiments have to determine if other important parameters of microglial activation, like cytokine expression and phagocytosis, are regulated autonomously or converge toward a common profile.

Conceptually, LPA can leak into the central nervous system during impairment of the blood-brain barrier. Blood-brain barrier impairment may occur rapidly (46) in diverse disorders, including stroke, trauma, inflammation, and seizures (47, 48). Indeed, it was shown that LPA-like activity in the cerebrospinal fluid increases after traumatic brain injury (23). LPA leaking into the central nervous system after blood-brain barrier impairment or due to LPA-induced increased tight junction permeability in cerebral endothelium (24) might be a signal leading to microglial activation. In contrast to cytokines, which are known to activate microglia, but need to be produced by cells, LPA would be immediately present at the site of injury.
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