Note

Lysophosphatidylcholine Potentiates BDNF-Induced TrkB Phosphorylation and Downstream Signals in Cerebellar Granule Neurons

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We found that brain-derived neurotrophic factor (BDNF)-induced phosphorylation of mitogen-activated protein kinase (MAPK) and Akt in cerebellar granule neurons was specifically potentiated by LPC. LPC also augmented the BDNF-induced phosphorylation of TrkB, the receptor for BDNF. In TrkB-transfected CHO-K1 cells, LPC potentiated BDNF-induced MAPK phosphorylation. These results suggest that LPC plays a role in BDNF-TrkB signaling by regulating the activation of TrkB.

Key words: Akt; brain-derived growth factor; cerebellar granule neurons; lysophosphatidylcholine; mitogen-activated protein kinase

Nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3, and neurotrophin-4 constitute a family of closely related small proteins that support and regulate the survival, development, and functions of neurons in the nervous system of vertebrates.1–3) BDNF is abundantly expressed in the developing and adult mammalian brains, and has been implicated in the pathophysiology of various brain diseases. The cellular actions of BDNF are mediated through two receptors, TrkB (high affinity) and p75 (low affinity). By binding to TrkB, BDNF induces the phosphorylation of TrkB and its kinase activity. Phosphorylated TrkB at tyrosine 515 activates the Ras-mitogen-activated protein kinase (MAPK) signaling pathway, which induces the phosphorylation of cAMP response element-binding protein and various transcription factors involved in cell survival, as well as the phosphatidylinositol 3-kinase (PI3K)-Akt signaling cascade, which inhibits proapoptotic signals, thereby promoting the survival of cells. Activated TrkB at tyrosine 816 recruits and phosphorylates phospholipase C-1 (PLC-γ-1), and this induces Ca\(^{2+}\)/calmodulin-dependent kinase (Ca\(^{2+}\)/CaM) activation by mobilizing Ca\(^{2+}\) from the intracellular stores to the cytoplasm, which eventually promotes cell survival.

Cerebellar granule neurons (CGNs) are the most abundant neurons in the brain. Cultured CGNs are a suitable model for studying cell survival and differentiation.4,5) BDNF and IGF-1 have been found to protect CGNs from low potassium-induced apoptosis by activating the Ras-MAPK and the PI3K-Akt signaling cascades, respectively.4,6) IGF-1 also induces activation of the t-type voltage-dependent Ca\(^{2+}\) channel and Ca\(^{2+}\)/CaM activation, promoting the survival of CGNs.7) LPC is known to be a bioactive lipid derived mainly from phosphatidylcholine in the plasma membrane and lipoprotein through the enzymatic action of PLA\(_2\).8) Diverse biological functions of LPC have been identified, including neurotrophin-like activity.9,10) We have reported that LPC significantly enhanced NGF-induced MAPK and Akt phosphorylation by stimulating the activation of TrkA in PC12 and CHO-K1 cells through the extracellular domain of TrkA.11) In the present study, we aimed to determine whether LPC has a similar effect on the BDNF-TrkB pathway. This might be important in the attempt to understand the signaling mechanism of neurotrophin-like activity elicited by LPC. We found that LPC promoted BDNF-induced MAPK phosphorylation in TrkB-transfected CHO-K1 cells. LPC also enhanced BDNF-induced but not IGF-1-induced MAPK and Akt phosphorylation in CGNs that endogenously express TrkB. These findings imply a specific interaction of LPC with TrkB.

LPC used in this study was 1-palmitoyl-sn-glycero-3-phosphocholine (C16:0; cat. no. 855675P; Avanti Polar Lipids, USA). Brain-derived neurotrophic factor (BDNF, PT45002) was purchased from Toyobo (Japan). Recombinant human insulin-like growth factor-1 (IGF-1, GPT-10011L) was purchased from Pepro Tech (USA). The primary antibodies used were phospho-p44/42 (Thr202/Tyr204) MAP kinase, #9101; p44/42 MAP kinase, #9102; phospho-Akt (Ser473), #9271; Akt, #9272; phospho-TrkA (Tyr490), #9141, and Trk-antibodies. All these antibodies were purchased from Cell Signaling Technology (USA). Living colors A.v. mononclonal antibody (JL-8) (#632380; Clontech, USA) was used at a dilution of 1:5000 in Tris-buffered saline (TBS). The secondary antibody, horseradish peroxidase-linked anti-rabbit-IgG (#7074; Cell Signaling Technology, USA), was used at a dilution of 1:2000 in TBS. Peroxidase-labeled anti-mouse IgG (H + L) (#PI-2000; Vector, USA) was used at a dilution of 1:500 in TBS.

Cerebellar granule neurons (CGNs) were obtained by dissociating the cerebella of mice on postnatal day 7 (P7). They were cultured in DMEM containing 10% fetal calf serum and 25 mM KCl in 0.2% polyethyleneimine-coated 24-well plates. The cells were maintained at 37 °C in 10% CO\(_2\) in a humidified incubator. After 24 h, the culture medium was exchanged for DMEM containing 10% FCS, 25 mM KCl, and 10 μM cytosine arabinoside, and the cells were cultured for 48 h. The medium was then exchanged for DMEM containing 10% FCS and 25 mM KCl, and the cells were incubated for 24 h before use.
for another 48 h. Five days after the culture was prepared, the cells were subjected to various treatments.

Cell lysates were collected in the sodium dodecyl sulfate (SDS) sample buffer at 50 μL/well and boiled for 3 min. In each experiment, 18 μL of cell lysate containing 20–30 μg proteins was subjected to electrophoresis on SDS gel. The proteins were transferred to a polyvinylidene fluoride membrane, which was incubated overnight at 4 °C with the primary antibody, as described above. After incubation with the secondary antibody, immunoreactive bands were visualized by SuperSignal® West Pico Lumino/Enhancer (#1856136) and SuperSignal® West Pico Stable Peroxide (#1856135) solution (Thermo Scientific, USA), or Western Lightning® Ultra solutions (PerkinElmer, USA). The amounts of protein were quantified by Multi Gauge software (Fuji Film, Japan). Each figure shows a representative result from two to three independent experiments that gave essentially the same result.

To construct a plasmid for expression of the TrkB-EGFP fusion protein, a cDNA fragment encoding full-length mouse TrkB (821 amino acid-long) was amplified by PCR and cloned into the EcoRI site of the pEGFP-N1 vector. CHO-K1 cells seeded in 24-well plate (>80% confluent) were transiently transfected with various plasmids (0.8 μg/well) over 18–24 h by Lipofectamine™2000 (1.5 μL/well; Invitrogen, USA) following the manufacturer’s instructions.

In the naive or vector-transfected CHO-K1 cells that did not express TrkB, no phosphorylation of MAPK (Erk1 and Erk2; 44 and 42 kDa, respectively) was observed under treatment with BDNF and/or LPC (data not shown). When TrkB was transfected to CHO-K1 cells, phosphorylation of MAPK was observed (Fig. 1A and B, lane 2), presumably due to the autophosphorylation of overexpressed TrkB. BDNF significantly increased MAPK phosphorylation (lane 4), suggesting that exogenously-expressed TrkB is functional. This response was significantly elevated when LPC (1 μM) was added together with BDNF (lane 5), while LPC added alone did not affect MAPK phosphorylation (lane 3). This suggests that LPC displays a similar effect on BDNF-TrkB signaling, in addition to its potentiation of NGF-TrkA signaling.

Since the BDNF-induced Ras-MAPK pathway plays a pivotal role in the survival of CGNs, and LPC was found to support the survival of CGNs similar to BDNF, a primary culture of CGNs from mouse cerebella that express endogenous TrkB as well as the receptor for IGF-1 was used. After it was serum-starved for 3 h in a low potassium medium, the effect of LPC on MAPK phosphorylation was tested in CGNs treated with and without BDNF (Fig. 2A). BDNF induced the phosphorylation of MAPK (lane 4), and we found that this phosphorylation was enhanced by co-addition of LPC (lane 5), while LPC added alone failed to induce MAPK phosphorylation (lane 3). Increasing the dose of LPC to 10 μM did not further enhance BDNF-induced MAPK phosphorylation (data not shown).

Since the Ras-MAPK cascade can also be activated by IGF-1 through its receptor,3 the question whether LPC enhances MAPK phosphorylation induced by IGF-1 was examined in CGNs. IGF-1 triggered the phosphorylation of MAPK (Fig. 2B, lane 3), but it was not further elevated by LPC (lane 4). These results indicate that LPC specifically stimulates MAPK phosphorylation triggered by BDNF, but not that induced by IGF-1 in CGNs.

We proceeded to examine the effect of LPC on another pathway induced by BDNF and IGF-1, Akt phosphorylation. Compared to the Akt phosphorylation induced by LPC or BDNF alone, Akt phosphorylation in the cells treated with LPC plus BDNF was significantly elevated (Fig. 2C, lanes 3, 4, and 5). In contrast, IGF-1-induced Akt phosphorylation (Fig. 2D, lane 3) was not affected by LPC (Fig. 2D, lane 4). Taken together, these results indicate that LPC is effective for BDNF-induced but not IGF-1-induced MAPK and Akt signals in CGNs.

To determine whether the increase in BDNF-induced phosphorylation of MAPK and Akt by LPC was a consequence of enhanced activation of TrkB, the receptor for BDNF, TrkB phosphorylation in CGNs treated with BDNF and/or LPC was analyzed. Treatment of CGNs with BDNF at 5 ng/mL induced modest phosphorylation of TrkB (Fig. 3, lane 4). When LPC was added together with BDNF, a significant increase in BDNF-induced TrkB phosphorylation was observed (lane 5), indicating that LPC potentiates BDNF-induced MAPK and Akt signaling pathways, presumably by enhancing the activation of TrkB.

Based on our previous observation that LPC and sPLA₂ display neurotrophin-like activities, we further analyzed the effect of LPC on BDNF-induced signals.

Fig. 1. LPC Enhanced BDNF-Induced MAPK Phosphorylation in TrkB-Transfected CHO-K1 Cells

A, CHO-K1 cells were untransfected (−) or transiently transfected with TrkB-pEGFP-N1 (TrkB-EGFP), and were grown for 18–24 h. They were then serum-starved for 1.5 h and treated with vehicle control, BDNF (5 ng/mL), LPC (1 μM), or BDNF together with LPC for 10 min. Phosphorylated and total MAPK (p-MAPK and t-MAPK) were quantified by Western blotting with anti-phospho-p44/42 MAPK and anti-44/42 MAPK antibodies respectively. Expression of TrkB-EGFP was detected by anti-GFP-antibody. The sizes (molecular mass/kDa) of full-length TrkB-EGFP (170) and the putative degradation product (155) are marked on the right. B, The amounts of p-MAPK and t-MAPK were quantified, and the ratio of p-MAPK vs t-MAPK was calculated. Data are means ± SD for three independent experiments. *p < 0.05 by one-way ANOVA.
We found that LPC promotes BDNF-induced activation of receptor TrkB and the phosphorylation of downstream signals, MAPK and Akt, which are known to be critical to the survival of CGNs. In contrast, LPC did not affect the IGF-1-induced MAPK and Akt signals in the CGNs. Although LPC added alone at 1 μM or together with BDNF failed to affect the low potassium-induced apoptosis of CGNs (data not shown), the findings of this study imply the existence of a specific role of LPC in the BDNF-TrkB signaling pathway.

Previously, intravenously administered LPC was found to enter the brain and to protect neurons from cell death in an in vivo ischemia model of mice, and more than half of LPC was found to be in unmetabolized form. Since BDNF is expressed abundantly in the nervous system, it can be argued that LPC entered to the brain and potentiated BDNF-induced MAPK and Akt signaling, thereby protecting neurons from apoptosis. LPC was effective even when injected 30 min after the onset of ischemia, and pretreatment with LPC 1 to 3 d before ischemia also showed a neuroprotective effect, suggesting that LPC is a promising therapeutic tool in treating ischemia-induced neuronal insult.

The molecular mechanism whereby LPC potentiates the BDNF-TrkB signal remains unknown. One possibility is that LPC affects the formation of the TrkB dimer. However, our recent work using TrkA-transfected CHO-K1 and PC12 cells indicates that the extracellular domain of TrkA is responsible for the effect of LPC, and that the formation of the TrkA dimer is not influenced by LPC. In a manner similar to LPC, a sialic acid-cholesterol amide conjugate, MCC-257, has been found to augment NGF-induced MAPK and Akt signals. Since this compound resembles cholesterol structurally, the authors speculate that it might influence the fluidity or the formation of microdomains in the plasma membrane, thereby affecting the activity of TrkA. In a similar scenario, LPC might modulate the physicochemical properties of the plasma membrane due to its amphiphilic nature, which in turn would affect the binding affinity of NGF to TrkA and of BDNF to TrkB. Further study is required to elucidate the precise mechanism of LPC-mediated enhancement of Trk receptor signals.

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References