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The Release of Glutamate from Cortical Neurons Regulated by BDNF Via the TrkB/Src/PLC-γ1 Pathway

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ABSTRACT

The brain-derived neurotrophic factor (BDNF) participates in the regulation of cortical neurons by influencing the release of glutamate. However, the specific mechanisms are unclear. Hence, we isolated and cultured the cortical neurons of Sprague Dawley rats. Specific inhibitors of TrkB, Src, PLC-γ1, Akt, and MEK1/2 (i.e., K252a, PP2, U73122, LY294002, and PD98059, respectively) were used to treat cortical neurons and to detect the glutamate release from cortical neurons stimulated with BDNF. BDNF significantly increased glutamate release, and simultaneously enhanced phosphorylation levels of TrkB, Src, PLC-γ, Akt, and Erk1/2. For BDNF-stimulated cortical neurons, K252a inhibited glutamate release and inhibited the phosphorylation levels of TrkB, Src, PLC-γ, Erk1/2, and Akt ($P < 0.05$). PP2 reduced the glutamate release from BDNF-stimulated cortical neurons ($P < 0.05$) and inhibited the phosphorylation levels of TrkB and PLC-γ1 ($P < 0.05$). However, PP2 had no effect on the phosphorylation levels of Erk1/2 or Akt ($P > 0.05$). U73122 inhibited the glutamate release from BDNF-stimulated cortical neurons, but had no influence on the phosphorylation levels of TrkB, Src, Erk1/2, or Akt ($P > 0.05$). LY294002 and PD98059 did not affect the BDNF-stimulated glutamate release and did not inhibit the phosphorylation levels of TrkB, Src, or PLC-γ1. In summary, BDNF stimulated the glutamate release from cortical neurons via the TrkB/Src/PLC-γ1 signaling pathway. J. Cell. Biochem. 114: 144–151, 2013.

KEY WORDS: CORTICAL NEURONS; GLUTAMATE; Src; TrkB; BRAIN-DERIVED NEUROTROPHIC FACTOR; PHOSPHOLIPASE C GAMMA 1

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, is an important signaling molecule that regulates the development and homeostasis of the central nervous system in higher animals via tropomyosin-related kinase (Trk) receptors. BDNF is largely considered to promote neuronal differentiation, plasticity, and survival. The interaction of BDNF with TrkB triggers receptor dimerization, autophosphorylation of intracellular tyrosine residues, and the subsequent activation of signaling pathways. The BDNF/TrkB signaling complex enters into neurons by endocytosis. Transport vesicles are then formed, which continue to regulate multiple signaling pathways, participating in the control of glutamate release [Lewin and Barde, 1996; Takei et al., 1998]. When the BDNF acts on cortical neurons for only a short time (e.g., 1 min), it promotes the temporary and rapid release of glutamate through the calcium channel pathway with the participation of glutamate transporters [Takei et al., 1998; Numakawa et al., 2002]. When the BDNF acts on cortical neurons over a long time (e.g., 24 h), it promotes the release of glutamate via the protein synthesis pathway by nuclear transcription [Matsumoto et al., 2006]. In addition, cholesterol stimulates the activity of TrkB in the lipid raft and dexamethasone decreases the interaction between TrkB and glucocorticoid receptors; both regulate the BDNF-stimulated glutamate release [Suzuki et al., 2004; Numakawa et al., 2009]. Although there have been many studies regarding the enhancement of BDNF-regulated glutamate release of neurons, the underlying mechanisms are still unclear.

Recent research suggests that there are interrelations between BDNF and Src in the regulation of nervous system function. Src, as a member of the non-receptor tyrosine kinase family, is widely expressed in nervous tissues such as the cerebral cortex, hippocampus and spinal cord, and hence plays an important part in the regulation of neurotransmitter release [Wiesner and Fuhrer, 2006]. In hippocampus neurons, BDNF participates in the regulation of memory formation through the combined increased activation for Src and TrkB [Mizuno et al., 2003]. Ohnishi et al. [2001] suggested that Src inhibits the dopamine release of PC12 cells by regulating

Zitao Zhang and Jin Fan contributed equally to this work.

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dynamic changes in actin. When linoleic acid is used to stimulate the round-shaped papilla taste buds in rat tongues, Src evokes the release of 5-hydroxytryptamine and noradrenalin by calcium signaling [El-Yassimi et al., 2008]. The mechanism of Src in regulating the release of glutamate is also complex. Wang [2003] found that Src promoted the release of glutamate in cortical neurons by regulating the recombination of cytoskeletal proteins. However, Baldwin et al. [2006] established that the specific inhibitor of Src, namely PP2, enhanced the exocytosis, and increased the release of glutamate. In glial cells, BDNF induces long-term potentiation (LTP) in the spinal cord via the activated Src, whereas PP2 inhibits the BDNF-induced LTP in the spinal cord [Zhou et al., 2011]. LTP and memory formation are both related to the release of glutamate. Thus, we speculate that Src plays an important role in BDNF-induced glutamate release.

PLCγ1 is a member of the PLC serine/threonine family. The phosphorylation of tyrosine 783 activates its enzyme activity and regulates the corresponding cell effect [Carpenter and Ji, 1999]. In cortical neurons, PLCγ1-tyr783 can be activated by BDNF [Widmer et al., 1993]. The activated PLCγ1 can regulate the plasticity of synapses and the development of neurons. Numakawa et al. [2001, 2002] found that in cerebellum neurons and cerebrum cortical neurons, the activity of PLCγ1 participates in the BDNF-induced glutamate release. Although there is a mutual regulation between Src and PLCγ1 [Haendeler et al., 2003; Kim et al., 2006; Shen et al., 2007], their interaction in BDNF-induced glutamate release still needs further research.

Our study showed that, for BDNF-stimulated cortical neurons, TrkB triggered the activity of Src. Following activation, Src had a positive feedback effect, promoting the full activation of TrkB through transmitting the signal to PLCγ1 by Src. It was the activated PLCγ1, and not Erk1/2 and Akt, which stimulated the glutamate release from cortical neurons.

MATERIALS AND METHODS

REAGENTS

Anti-rat Src, p-Src^{tyr416}, PLCγ1, p-PLCγ1^{tyr783}, Erk1/2, p-Erk1/2$^{thr202/tyr204}$, and Akt, p-Akt^{ser473} antibodies, as well as PD98058 and LY294002, were purchased from Cell Signaling (Danvers, MA). TrkB and β-tubulin III were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). 4G10 and NeuN were from Millipore (Billerica, MA). All secondary antibodies used in this study were purchased from the Beyotime Institute of Biotechnology (Songjiang, Shanghai, China). K252a, PP2, and U73122 were from Merck (Darmstadt, Germany). BDNF was from PeproTech (Rocky Hill, NJ).

THE CULTURE AND IDENTIFICATION OF RAT PRIMARY CORTICAL NEURONS

In accordance with the improved methods of Takei and Endo [Takei and Endo, 1994; Takei et al., 1998], cortical neurons were isolated from Sprague Dawley rat embryos at the 18th day of gestation (E18) and cultured. Briefly, the E18 embryos were dissociated under aseptic conditions. After splitting the meninges, the cortices were dissected and digested with Trypsin/DNase I for 20 min. Cells were cultured in a polylysine pre-coated 12-well plate, with a density of $5 \times 10^5/cm^2$ in each well. Half of the medium was replaced with fresh Neurobasal culture medium (containing 2% B27, 1% glutamine, 100 IU/ml penicillin, and 100 mg/ml streptomycin) every 3 days. On the 6th day (DIV 6) of culturing, two specific antibodies, β-tubulin III and NeuN, were used to carry out immunofluorescence identification in the cells. After the identification, the relevant experiments were performed in the cells during the same period. Various inhibitors were added into the medium 30 min before the stimulation of BDNF (100 ng/ml). The concentrations of PP2, K252a, PD98059, LY294002, and U73122 were 10 μM, 200 nM, 25 μM, 50 μM, and 5 μM, respectively. An equal amount of dimethyl sulfoxide (DMSO) was incubated with the cells as a solvent control.

DETECTION OF GLUTAMATE CONTENT

After the stimulation of BDNF-treated (100 ng/ml) cortical neurons (DIV 6) for 1 min, the culture medium was examined immediately. A glutamate (colorimetric) assay kit (Biovision, Milpitas, CA) was used to determine the glutamate content in the culture medium as described in a previous study [Tao et al., 2011].

WESTERN BLOT ANALYSIS

Total cellular extracts were obtained by performing cell lysis using an RIPA buffer (Beyotime Institute of Biotechnology, Songjiang, Shanghai, China). Protein concentration was determined using the Bradford method. Aliquots of cell lysates were separated by 12% SDS-polyacrylamide gel, and transferred to polyvinylidene fluoride membranes (Millipore, Billerica, MA). The membranes were incubated overnight with the corresponding antibody at 4°C. This was followed by the addition of a horseradish peroxidase-linked secondary antibody, and electrochemiluminescence visualization of the bands (Beyotime Institute of Biotechnology, Songjiang, Shanghai, China). Quantification of the bands was carried out using Quantity One (Bio-Rad) densitometric analysis software.

STATISTICAL ANALYSIS

Experimental data were expressed as mean ± SEM. All experiments were repeated three times independently. Student's *t*-tests or one-way ANOVA was used to compare difference among the groups. A *P*-value of less than 0.05 was considered statistically significant.

RESULTS

BDNF PROMOTED THE GLUTAMATE RELEASE FROM CORTICAL NEURONS

As shown in Figure 1, the results for immunofluorescence staining were positive for both β-tubulin III and NeuN (DIV 6). In contrast to the control group, BDNF promoted the release of glutamate ($P < 0.05$). Moreover, when the concentration of BDNF was 100 ng/ml, the effect on glutamate release was higher than when the concentration of BDNF was 50 ng/ml. However, there was no significant difference between the effect of BDNF with a concentration of 150 ng/ml and BDNF with a concentration of 100 ng/ml on promoting the glutamate release (Table I). Therefore, subsequent experiments used the concentration of 100 ng/ml BDNF as a working concentration.

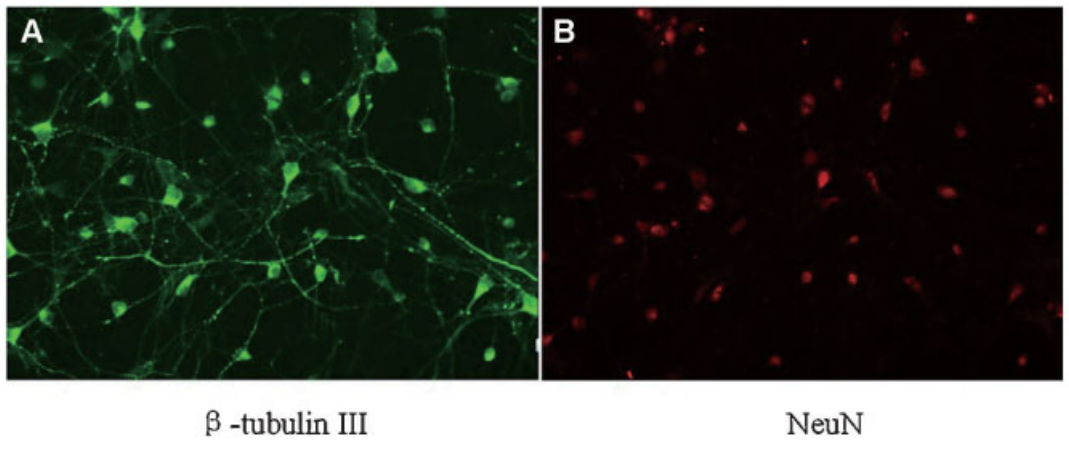

Fig. 1. The isolation and culture of neurons using the antibodies β-tubulin III and NeuN for immunofluorescence staining. A: β-tubulin III staining—cells were shown to be green by the FITC marker. B: NeuN staining—cells were shown to be red by the rhodamine tags.

FOR BDNF-STIMULATED CORTICAL NEURONS, THE ACTIVITY OF Src, TrkB, Erk1/2, PLCγ1, AND Akt INCREASED

In order to confirm the interrelation of protein kinases for the BDNF-stimulated glutamate release in signaling pathways, we detected the phosphorylation levels of TrkB, Src, PLCγ1, Erk1/2, and Akt separately. BDNF (100 ng/ml) was used to stimulate cortical neurons for 0 s, 30 s, 2 min, 5 min and 15 min, individually. Statistical results revealed that TrkB, Src, Erk1/2, PLCγ1, and Akt were quickly activated following the BDNF stimulation (Fig. 2). Hence, it was possible that TrkB, Src, Erk1/2, PLCγ1, and Akt all participated in the BDNF-regulated glutamate release.

FOR BDNF-STIMULATED CORTICAL NEURONS, TrkB PARTICIPATED IN THE RELEASE OF GLUTAMATE AND Src ACTIVATION

As an important member of the nerve growth factor family, BDNF has a corresponding cell effect by combining with TrkB or p75NTR receptors on the cell membrane. However, in different neurons, BDNF induces the activity of Src via different receptors. For instance, in hippocampus neurons, it is via the TrkB pathway [Huang and McNamara, 2010], while in Schwann cells, it is via the p75NTR pathway [Yamauchi et al., 2004]. Western blot results revealed that there were expressions of both TrkB and p75NTR in cortical neurons (Fig. 3). In order to further confirm the upstream signal molecules of Src in the BDNF signaling pathway for cortical neurons, we used the specific inhibitors K252a of TrkB to pre-treat cortical neurons. The results showed that K252a significantly inhibited the BDNF-induced glutamate release from cortical neurons (Table II) and inhibited Src phosphorylation (Fig. 4B and F). These results indicate that BDNF induced the Src phosphorylation via TrkB in cortical neurons, and thus promoted the glutamate release from cortical neurons. Although K252a inhibited the Src phosphorylation, this does not exclude the effect of the p75NTR pathway on Src activation.

TABLE I. Effects of Different Concentrations of BDNF on the Glutamate Release From Cortical Neurons

	Control	BDNF (50 ng/ml)	BDNF (100 ng/ml)	BDNF (150 ng/ml)
Glutamate level (nmol/ml)	$3.51 \pm 0.17^{*}$	$6.28 \pm 0.11^{**}$	8.74 ± 0.22	8.92 ± 0.26

$^{*}P < 0.05$ in contrast with 50 ng/ml group.
$^{**}P < 0.05$ in contrast with 100 ng/ml group.

Src PARTICIPATED IN THE GLUTAMATE RELEASE FROM BDNF-STIMULATED CORTICAL NEURONS

As a member of the non-receptor tyrosine kinase family, Src participates in regulating the release of neurotransmitters, although there is still controversy regarding the underlying mechanisms. To define the effect of Src on the BDNF/TrkB-mediated glutamate release, we used the Src inhibitor, PP2, to pre-treat cortical neurons. The results of the western blot showed that PP2 inhibited Src phosphorylation (Fig. 5A and B), and it significantly inhibited the BDNF-induced glutamate release (Table II). This result indicates that Src participated in the BDNF/TrkB-regulated glutamate release, inhibited the activity of Src, and reduced the release of glutamate. However, this does not exclude the possibility that BDNF might promote the release of glutamate via other pathways.

FOR BDNF-STIMULATED CORTICAL NEURONS, Src CONTRIBUTED TO THE ACTIVATION OF TrkB AND PLCγ1

To confirm whether TrkB was also regulated by Src, we used the Src inhibitor, PP2, to pre-treat cortical neurons. The results suggest that PP2 inhibited the phosphorylation and glutamate release of TrkB receptors (Fig. 6A and E). This indicates that although Src was as a downstream signal molecule of TrkB, it also could regulate the activity of TrkB with positive feedback, promote its full activation, and participate in regulating the signal transmission of BDNF/TrkB. Erk1/2, Akt, and PLCγ1 were important kinases in the BDNF/TrkB pathway. In order to confirm the interrelations between Src in the process of BDNF stimulation of the cortical neurons and the above

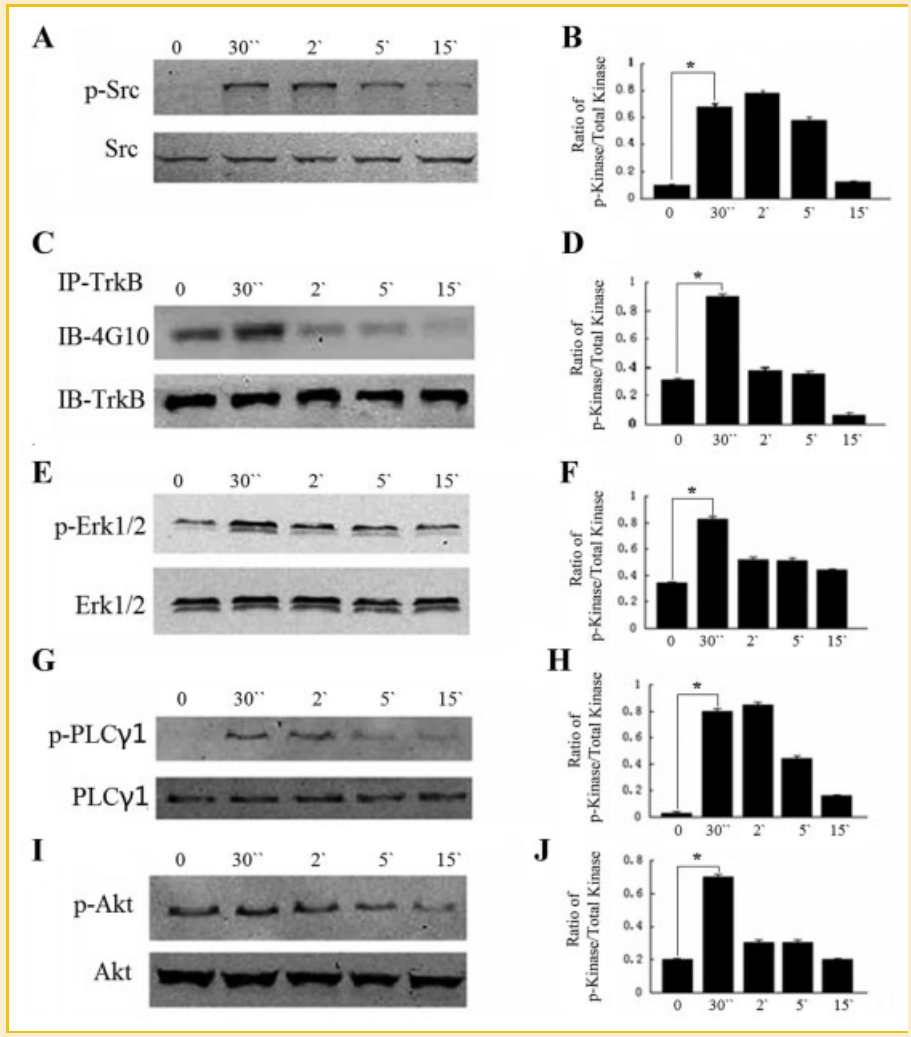

Fig. 2. Effects of BDNF on Src, TrkB, Erk1/2, PLCγ1, and Akt phosphorylation. A: Effects of BDNF stimulation on Src phosphorylation at 0 s, 30 s, 2 min, 5 min, and 15 min. B: Statistical results of (A) ($^*P < 0.05$ when compared with the control group). C: Effects of BDNF stimulation on TrkB phosphorylation at 0 s, 30 s, 2 min, 5 min, and 15 min. D: Statistical results of (C) ($^*P < 0.05$ when compared with the control group). E: Effects of BDNF stimulation on Erk1/2 phosphorylation at 0 s, 30 s, 2 min, 5 min, and 15 min. F: Statistical results of (E) ($^*P < 0.05$ when compared with the control group). G: Effects of BDNF stimulation on PLCγ1 phosphorylation at 0 s, 30 s, 2 min, 5 min, and 15 min. H: Statistical results of (G) ($^*P < 0.05$ when compared with the control group). I: Effects of BDNF stimulation on Akt phosphorylation at 0 s, 30 s, 2 min, 5 min, and 15 min. J: Statistical results of (I) ($^*P < 0.05$ when compared with the control group).

kinases, we examined the activity of Erk1/2, Akt, and PLCγ1. The results showed that the Src inhibitor, PP2, had a significant inhibition effect on the phosphorylation level of PLCγ1 (Fig. 6C and E), but had no obvious influence on the phosphorylation levels of Erk1/2 or Akt (Fig. 6B, D, and E). These results suggest that although Erk1/2 and Akt participated in the signal transmission of BDNF/TrkB, they did not participate in the BDNF/TrkB/Src-regulated glutamate release from cortical neurons.

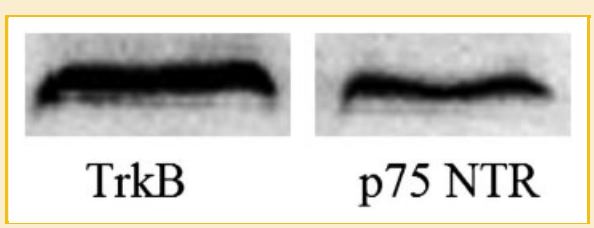

Fig. 3. The expression of TrkB and p75 NTR in cortical neurons. TrkB and p75 NTR were both shown to be expressed in cortical neurons (DIV 7) by western blotting.

THE EFFECTS OF Erk1/2, AKT, AND PLCγ1 ON Src ACTIVITY AND GLUTAMATE RELEASE FROM BDNF-STIMULATED CORTICAL NEURONS

The above findings show that Src regulated the activity of PLCγ1, but had no obvious effect on the activity of Erk1/2 or Akt. To further confirm the interrelations among Erk1/2, Akt, PLCγ1, and Src, we used PD98059 (MEK1/2 specific inhibitor), LY294002 (Akt inhibitor) and U73122 (PLCγ1 inhibitor), respectively, to observe their effect on other corresponding protein kinases and on glutamate release. The results showed that U73122 inhibited the release of glutamate, whereas it had no effect on the activity of Erk1/2, Akt, or Src.

TABLE II. The Effects of TrkB, Src, PLC-γ1, Akt, and Erk1/2 on Glutamate Release From BDNF-Stimulated Cortical Neurons

	K252a	PP2	PD98059	U73122	LY290024	DMSO
Glutamate level (nmol/ml)	$4.02 \pm 0.36^*$	$6.06 \pm 0.69^*$	7.96 ± 0.32	$3.87 \pm 0.33^*$	8.12 ± 0.11	8.08 ± 0.16

K252a (200 nM), PP2 (10 μM), U73122 (5 μM), LY294002 (50 μM), or PD98059 (25 μM) were individually added into the culture medium 30 min before the BDNF stimulation, and their effects on glutamate release were examined.
$^*P < 0.05$ in contrast with DMSO-treated group.

PD98059 and LY294002 did not inhibit the release of glutamate, and had no remarkable influence on the activity of Src and PLCγ1 (Table II; Fig. 7A–F).

DISCUSSION

As a major excitatory neurotransmitter in the brain and spinal cord of mammals, glutamate has a diverse, but important role in the central nervous system of vertebrates. It is implicated in the transmission of synapses, cognitive functions relevant to the learning and memory [Kendell et al., 2005], neurotoxicity [Smith, 2000], and so on. The accuracy and appropriateness of glutamate release is a key factor in maintaining the normal physiological functioning of the nervous system and for avoiding neurotoxicity [Meldrum and Garthwaite, 1990]. BDNF is an important signal molecule in regulating the development and homeostasis of the central nervous system in higher animals. BDNF could stimulate the rapid release of glutamate through the intracellular, fast-activated signaling pathway via the phosphorylation of certain protein kinases.

Our main findings were that TrkB and Src promoted the activity of PLCγ1, via mutual regulation, and mediated the glutamate release from cortical neurons induced by BDNF. Although Erk1/2 and Akt participated in the signal transmission of BDNF/TrkB, they did not participate in the BDNF/TrkB/Src/PLCγ1-regulated glutamate release from cortical neurons.

Src, as a member of non-receptor tyrosine kinase families, is widely expressed in neurons and regulates the release of neurotransmitters via the phosphorylation of synapses' vesicle proteins such as synapsin I and synaptophysin [Wiesner and Fuhrer, 2006]. Nevertheless, there is still controversy with regard to the mechanism of Src in regulating the glutamate release of major excitatory neurotransmitters within the nervous system. Wang [2003] considered that Src promoted the release of glutamate via the influx of calcium ion. However, Baldwin et al. [2006] found that, after Src had been activated, it inhibited the release of glutamate. Recent studies have shown that Src, as a protein kinase, produces the corresponding cell effect by regulating some important downstream signal molecules, such as the transmission signals of PLCγ1, Erk1/2, or focal adhesion kinase [Kim et al., 2006; Shen et al., 2007; Meng et al., 2009]. Src also participates in the LTP and memory formation induced by BDNF [Mizuno et al., 2003; Zhou et al., 2011]. However, both LTP and memory formation are relevant to the release of neurotransmitters. Thus, we speculate that Src regulated the glutamate release through the control of some important signal molecules. The results from this study showed that the Src inhibitor, PP2, affected the cortical neurons, and significantly inhibited the

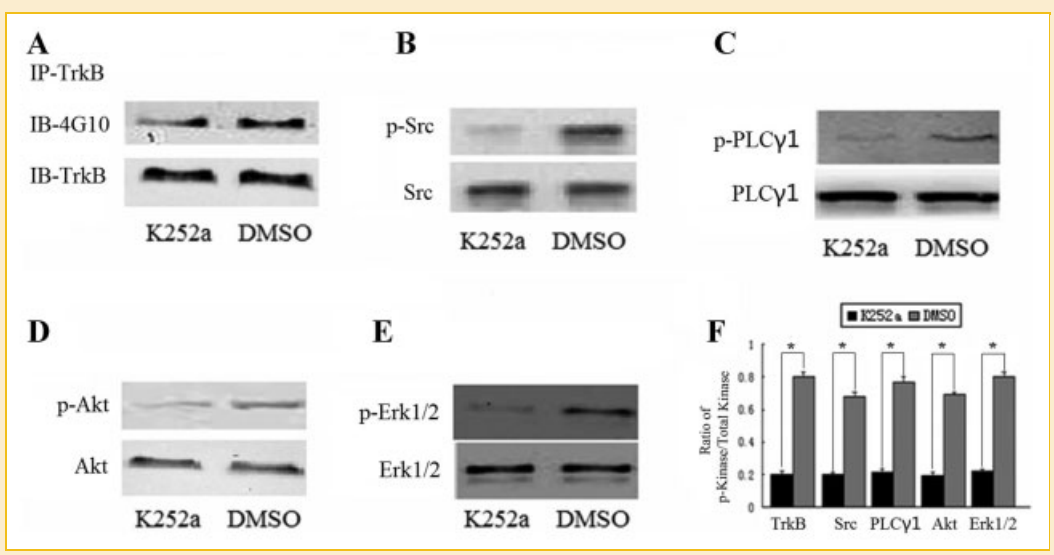

Fig. 4. Effects of K252a on TrkB, Src, PLCγ1, Akt, and Erk1/2 phosphorylation. A: K252a (200 nM) was added into the culture medium 30 min before the BDNF stimulation, and the effect of K252a on TrkB phosphorylation at 30 s was examined. B: The effect of K252a on Src phosphorylation at 30 s. C: The effect of K252a on PLCγ1 phosphorylation at 30 s. D: The effect of K252a on Akt phosphorylation at 30 s. E: The effect of K252a on Erk1/2 phosphorylation at 30 s. F: Statistical results of (A–E) ($^*P < 0.05$).

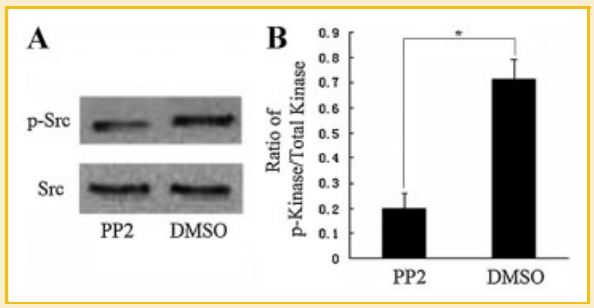

Fig. 5. The effect of PP2 on Src phosphorylation for BDNF-stimulated cortical neurons. A: PP2 (10 μM) was added into the culture medium 30 min before the BDNF stimulation, and the effect of PP2 on Src phosphorylation at 30 s was examined. B: Statistical results of (A) ($^*P < 0.05$).

release of glutamate. This suggests that Src partly contributed to the BDNF-induced glutamate release from cortical neurons.

The results from this study indicate the expression of two BDNF receptors in the cortical neurons of rats: TrkB and p75NTR (Fig. 3). Some research has shown that BDNF via either TrkB or p75NTR will promote the activity of Src [Mizuno et al., 2003; Yamauchi et al., 2004] and mediate the corresponding cell effect. To determine the effect of activated Src on the process of TrkB-induced glutamate release, we used K252a to inhibit the activation of TrkB. The results revealed that K252a could inhibit the TrkB activity induced by BDNF, and the Src activity was also inhibited. Although p75NTR was not inhibited directly, the results for this study show that the TrkB receptor pathway mediated the Src activity in the cortical neurons induced by BDNF. Furthermore, the Src activity was an important kinase for BDNF/TrkB-induced glutamate release from cortical neurons [Takei et al., 1998]. Moreover, when the Src activity was inhibited, the phosphorylation levels of TrkB and the glutamate release were also inhibited. Although not completely inhibited, it still indicates that Src cannot only be used as a TrkB downstream signal molecule but can also be used as its upstream signal molecule. When BDNF acted on TrkB, it activated Src. Following the activation, Src had a positive feedback effect, and promoted the full activation of TrkB, and was therefore beneficial to the glutamate release from cortical neurons (Fig. 7). Previous research has shown that there are interactions between Src and platelet-derived growth factor receptor, epidermal growth factor receptor, and fibroblast growth factor receptor in receptor-type protein tyrosine kinase families [Bromann et al., 2004]. Our study confirmed that TrkB, as a member of this family, also shared interactions with Src. In addition, Src was an important kinase when TrkB was involved in the glutamate release from cortical neurons.

For PLCγ1, a member of PLC serine/threonine family, the phosphorylation of tyrosine 783 activates its enzyme activity, transmits signals, and regulates the corresponding cell effect [Carpenter and Ji, 1999]. When the TrkB/PLCγ1 signaling pathway is intervened within the nervous system, the epileptic seizure of mice is inhibited [He et al., 2010]. Similarly, PLCγ1 affects the structural plasticity of sensory neurons and the dendrite formation of olfactorius bulbus' interneurons in the vestibular system [Berghuis et al., 2006; Sciarretta et al., 2010]. In the cerebellum neurons and cerebrum cortical neurons, the PLCγ1 activity participates in the glutamate release induced by BDNF [Numakawa et al., 2001, 2002]. Nevertheless, in the BDNF/TrkB signaling pathway, there is still controversy regarding how the PLCγ1 activity is regulated. In this study, we found that the inhibitors of Src and TrkB inhibited PLCγ1 activity, indicating that TrkB and Src, via mutual regulation,

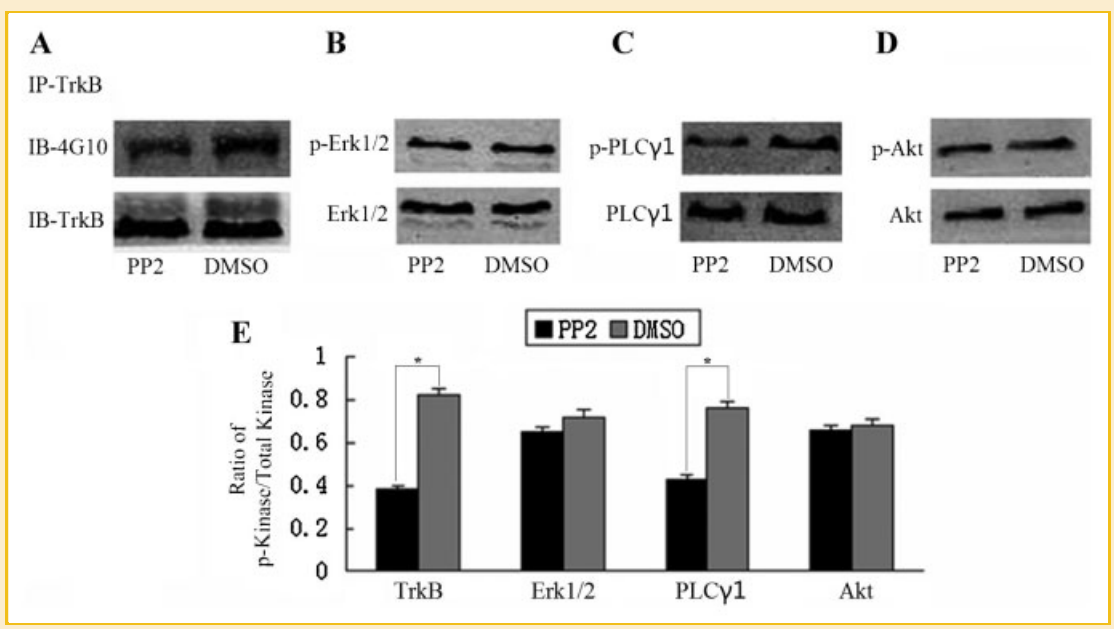

Fig. 6. The separate effects of PP2 on TrkB, Erk1/2, PLCγ1, and Akt activation for BDNF-stimulated cortical neurons. A: The effect of PP2 on TrkB phosphorylation at 30 s. B: The effect of PP2 on Erk1/2 phosphorylation at 30 s. C: The effect of PP2 on PLCγ1 phosphorylation at 30 s. D: The effect of PP2 on Akt phosphorylation at 30 s. E: Statistical results of (A–D). ($^*P < 0.05$).

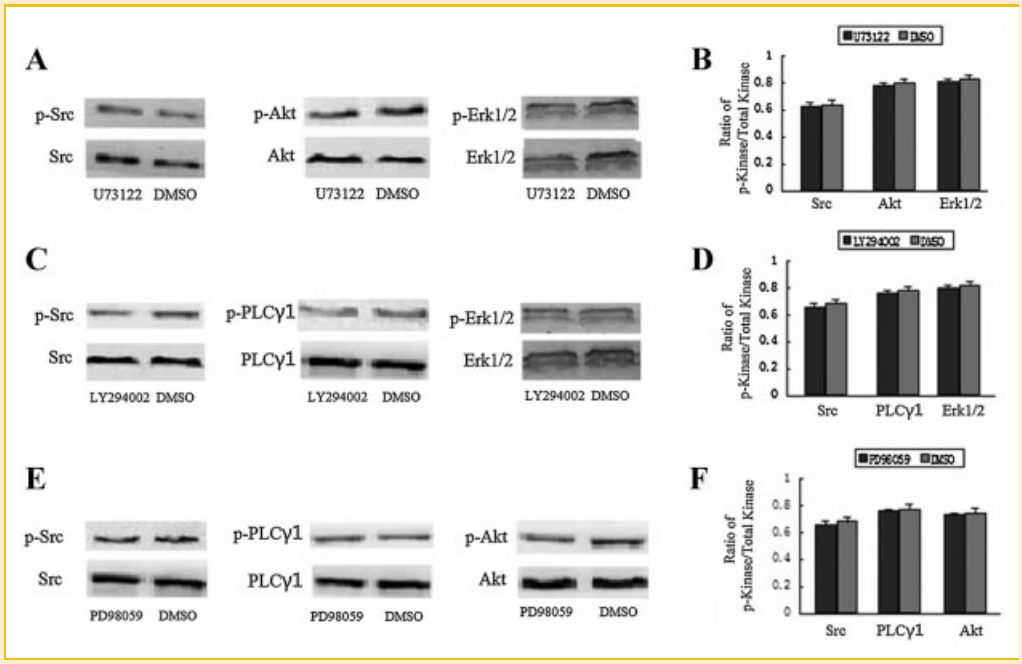

Fig. 7. The effects of U73122 (5 μM), LY294002 (50 μM), and PD98059 (25 μM) on the phosphorylation of other corresponding kinases for BDNF-stimulated cortical neurons. A: Effects of U73122 on Src, Akt, and Erk1/2 phosphorylation at 30 s. B: Statistical results of (A). C: Effects of LY294002 on Src, PLCγ1, and Erk1/2 phosphorylation at 30 s. D: Statistical results of (C). E: Effects of PD98059 on Src, PLCγ1, and Akt phosphorylation at 30 s. F: Statistical results of (E) ($^*P < 0.05$).

activated the PLCγ1 activity and mediated the BDNF-induced glutamate release from cortical neurons.

In the BDNF/TrkB signaling pathway, Erk1/2 and Akt were also activated (in addition to PLCγ1) [Slack et al., 2004]. Linoleic acid, via inhibiting the Erk1/2 activity, inhibits the glutamate release from cortical neurons, while Akt affects the release of catecholamines through its influence on glycogen synthase kinase 3 activation [El-Yassimi et al., 2008; Siraskar et al., 2011]. The relationship between PLCγ1, Erk1/2, and Akt is complex. In chronic myeloid leukemia cells, PLCγ1 and Akt can together promote the proliferation of leukemia cells. In the embryo fibroblast of mice, the activation of PLCγ1 regulates the activity of Akt and Erk1/2 [Amin et al., 2003; Markova et al., 2010]. The interrelations among PLCγ1, Erk1/2, and Akt in the BDNF/TrkB signaling pathway are still unclear. The findings of this study suggest that although the PLCγ1 inhibitor could inhibit the glutamate release from cortical neurons induced by BDNF, it had no obvious effect on the activity of Src, Erk1/2, or Akt. Inhibitors of Erk1/2 and Akt did not influence the BDNF-induced glutamate release from cortical neurons, and they had no remarkable effect on the activity of PLCγ1 or Src, indicating that there were no interactions among PLCγ1, Erk1/2, and Akt in the glutamate release from cortical neurons regulated by BDNF/TrkB. However, PLCγ1 did participate in the glutamate release from cortical neurons regulated by BDNF/TrkB/Src.

In summary, our study suggests that there was a mutual regulation of Src and TrkB on the BDNF-induced glutamate release from cortical neurons. For BDNF-stimulated cortical neurons, Src was activated by TrkB and it could promote the full activation of TrkB. Following the full activation, TrkB promoted the release of glutamate by increasing the level of PLCγ1 phosphorylation (Fig. 8).

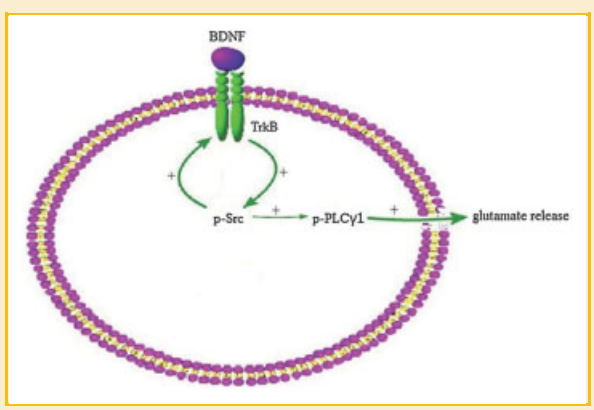

Fig. 8. BDNF via TrkB/Src/PLCγ1 promoted the glutamate release from cortical neurons, as shown in the schematic diagram. The combination of BDNF with TrkB receptors promoted the phosphorylation of TrkB, and the activation of Src. Following activation, Src had a positive feedback effect on TrkB, and triggered the full activation of TrkB, thus promoting the activity of PLCγ1 and the glutamate release from cortical neurons.

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