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*Original Article*

# *PKMζ* knockdown disrupts post-ischemic long-term potentiation via inhibiting postsynaptic expression of aminomethyl phosphonic acid receptors

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## Abstract

Post-ischemic long-term potentiation (i-LTP) is a pathological form of plasticity that was observed in glutamate receptor-mediated neurotransmission after stroke and may exert a detrimental effect via facilitating excitotoxic damage. The mechanism underlying i-LTP, however, remains less understood. By employing electrophysiological recording and immunofluorescence assay on hippocampal slices and cultured neurons, we found that protein kinase Mζ (PKMζ), an atypical protein kinase C isoform, was involved in enhancing aminomethyl phosphonic acid (AMPA) receptor (AMPAR) expression after i-LTP induction. *PKMζ* knockdown attenuated postsynaptic expression of AMPA receptors and disrupted i-LTP. Consistently, we observed less neuronal death of cultured hippocampal cells with *PKMζ* knockdown. Meanwhile, these findings indicate that PKMζ plays an important role in i-LTP by regulating postsynaptic expression of AMPA receptors. This work adds new knowledge to the mechanism of i-LTP, and thus is helpful to find the potential target for clinical therapy of ischemic stroke.

**Keywords:** PKMζ, i-LTP, AMPA receptor, oxygen glucose deprivation

## Introduction

The interruption of blood supply to the brain leads to ischemic stroke. As one of the leading causes of death and adult disability in the world, ischemic stroke draws more and more theoretical and clinical research[1]. However, the mechanisms underlying ischemic damage in the brain are still less understood. After stroke, glutamate receptor mediated pathological neural plasticity termed post-ischemic long-term potentiation (i-LTP) often occurs[2-3]. Although such neural plasticity plays roles in recovery[4], excessive calcium entry and related kinase responses involved facilitate excitotoxicity and further damages. Therefore, improving the understanding of mechanisms mediating i-LTP after stroke is urgent.

It is well known that AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid) -type glutamate receptors (AMPARs) mediate main neurotransmission in i-LTP[3]. AMPARs are heteromeric channels composed of GluA1-4 subunits. GluA2-containing AMPARs are calcium-impermeable, and AMPARs lacking GluA2 are calcium-permeable (CP-AMPARs)[5]. It is reported that GluA2-containing AMPARs are rapidly internalized after oxygen and glucose deprivation (OGD, a widely used ischemic model in vitro, mimicking the lack of oxygen

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and glucose in ischemic stroke), which are replaced by CP-AMPARs[6,7]. Excessive expression of CP-AMPARs in i-LTP results in more calcium and $Zn^{2+}$ entry and excitotoxicity during stroke[8-10]. In fact, inhibitors of AMPARs (such as NBQX) and special inhibitors of CP-AMPARs (such as NAPSM) can effectively prevent cell death due to ischemia[11]. Almost certainly, the persistent increase of CP-AMPAR expression after ischemia plays an important role in i-LTP-induced neuronal damage. Therefore, the mechanisms underlying the regulation of CP-AMPAR expression in i-LTP need to be addressed.

In physiological conditions, development, learning and environment challenges can also induce long-term changes of synaptic transmission. The postsynaptic membrane expression of AMPARs is also the key step in this kind of LTP. It has been demonstrated that many molecular mechanisms involved in physiological LTP are similar to those for pathological changes[4]. The postsynaptic expression of AMPARs is regulated by several protein kinases, such as protein kinase C (PKC), cAMP-dependent protein kinase A (PKA) and $Ca^{2+}$/calmodulin-dependent protein kinase II (CaMKII)[12]. In the past decade, PKMζ, a brain-specific autonomously active isozyme of PKC, has been studied extensively and regarded as the key molecule to maintain physiological LTP and many types of memories[13]. It sustained LTP by increasing the number of AMPARs at postsynaptic sites. In addition, PKMζ maintaining long-term changes of neurotransmission could mediate several neurological and psychiatric disorders, exemplified by chronic neuropathic pain[14-15] and post-traumatic stress disorder[16]. It is interesting to know whether PKMζ also participates in i-LTP and ischemia-induced enhancement in postsynaptic expression of AMPARs.

Hippocampal CA1 area is one of the most vulnerable regions[17-18] after global ischemic stroke. In this work, we employed electrophysiological recording and Western blotting assay on OGD treated hippocampal slices and cultured neurons. We observed that i-LTP was accompanied by enhancement of postsynaptic expression of GluA1 and PKMζ. By injecting short hairpin RNA (shRNA) targeting *PKMζ* delivered by lentiviruses, OGD-induced i-LTP was suppressed by *PKMζ* knockdown and the localization of GluA1 at postsynaptic sites was decreased. Furthermore, neuron survival after OGD was also increased after *PKMζ* knockdown. Our data deepens the knowledge of pathological plasticity after cerebral ischemia and provides useful experimental clues for stroke therapeutics.

## Materials and methods

### Hippocampal slice preparation

All animals and experimental protocols were carried out by the guidance of the National Institutes of Health for the Care and Use of Laboratory Animals. Male Sprague-Dawley (SD) rats (4-6 weeks old) were anesthetized with 10% chloral hydrate followed by decapitation. The entire brain was rapidly removed. Coronal hippocampal brain slices (350 μm) were prepared by a vibratome (VT1000S, Leica, Germany) in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mmol/L) 125 NaCl, 3.25 KCl, 2 $CaCl_2$, 25 $NaHCO_3$, 1.25 $NaH_2PO_4$ and 11 glucose (pH=7.4). The ACSF was saturated with 95% $O_2$ and 5% $CO_2$. And fresh slices were recovered in oxygenated ACSF at 34 °C for at least 1 hour before experiments.

### Intracranial virus injection

ShRNA (5′-ACATTAAGCTGACGGACTA-3′) targeting *PKMζ* (PKMζ-shRNA) and the scrambled shRNA (5′-CATGTAAGACGACTATGAC-3′, scr-shRNA) were packaged in lentiviruses (Neuron Biotech, Shanghai, China). For intracranial virus injections, male SD rats, 4-6 weeks old, were deeply anesthetized with 10% chloral hydrate. Then, rats were fixed on a stereotaxic frame (RWD Life Science, Shenzhen, Guangdong, China). The 10 μL syringe was stereotaxically targeted to the dorsal hippocampus CA1 region (AP: -4.56 mm relative to bregma; ML: ±3.0 mm; DV: 3.0 mm[19]). Injections were carried out at a rate of 1 μL/minute, and controlled by a microsyringe pump controller (WPI, Sarasota, FL, USA). The needle was left in place for 6 additional minutes after virus injection. Rats were allowed 7 days to recover before electrophysiological recording or Western blotting assays.

### Primary hippocampal cell culture and transfection

Primary hippocampal cultures were prepared from E18-19 rats as previously described[20] and grown in neurobasal medium supplemental with 2% B27 (Gibco) and 1% Glutamax (Life Technologies, Carlsbad, CA, USA). Neurons were grown on coverslips coated with poly-D-lysine (0.1 g/L, Sigma-Aldrich, St Louis, MO, USA) in 12-well plates and cultured in humidified atmosphere with 5% $CO_2$ at 37 °C. For transfection of PKMζ-shRNA and scrambled (scr)-shRNA, lentiviruses were added into wells at DIV8-10.

### Electrophysiological recordings

Field excitatory postsynaptic potential (fEPSP) recording on acute hippocampal slices were performed with Axopatch-200B/700B amplifier (Molecular Device, Sunnyvale, CA, USA) and recorded from the stratum radiatum of CA1 in ACSF perfusion medium containing bicuculline methiodide (10 μmol/L). OGD was applied by 95% $N_2$/5% $CO_2$ in an ACSF solution containing 11 mmol/L sucrose for 3 minutes.

Whole-cell recordings were made from DIV10 -14 cultured neurons as previously described[21-22]. Cells were voltage clamped at -65 mV. Patch pipettes were filled with electrolyte solution containing (in mmol/L) 140 CsCl, 2.5 EGTA, 2 $MgCl_2$, 10 HEPES, 2TEA and 4 $K_2ATP$ (pH=7.4). Cells were perfused continuously with extracellular solution (ECS) containing (in mmol/L) 140 NaCl, 1.3 $CaCl_2$, 5 KCl, 25 HEPES, 33 glucose, 1 $MgCl_2$, 0.01 bicuculline methiodide and 0.0005 TTX (pH=7.4). Miniature excitatory postsynaptic currents were continuously recorded using an Axopatch-700B amplifier and records were filtered at 2 kHz. OGD-induced i-LTP was made as the protocol on slice, anoxia/hypoglycemia by 95% $N_2$/5% $CO_2$ and ECS containing 33 mmol/L sucrose instead of glucose for 3 minutes.

### Western blotting assay

Hippocampal slices were prepared as described above. Slices were then incubated in ACSF at 34 °C (95% $O_2$ and 5% $CO_2$) for 40 minutes. After incubation, slices were treated with OGD for 3 minutes, and recovered in normal ACSF for 30 minutes. Then, slices were homogenized in cold 0.32 mol/L sucrose containing (in mmol/L) 1 HEPES, 1 $MgCl_2$, 1 $NaHCO_3$, 20 sodium pyrophosphate, 20 β-phosphogrycerol, 0.2 dithiothreitol, 1 EDTA, 1 EGTA, 50 NaF, 1 $Na_3VO_4$ and 1 p-nitrophenyl phosphate (PNPP, pH=7.4) in the presence of protease and phosphatase inhibitors. The homogenate was centrifuged at 1,000 × *g* for 10 minutes. The supernatant was collected for subsequent analysis.

For Western blotting assay, samples were heated for 5 minutes at 95 °C with 5 × SDS loading buffer, subjected to SDS-PAGE and transferred to a PVDF membrane. Then, they were blocked with 3% (w/v) BSA (fraction V) in Tris buffered saline Tween (TBST, 0.1% Tween 20) for 1 hour at room temperature and analyzed by immunoblotting with the following antibodies: mouse monoclonal anti-GluR1-NT antibody (MAB2263; Millipore, Billerica, MA, USA), rabbit monoclonal anti-PSD95 (N-term) antibody (04-1066; Millopore), rabbit polyclonal anti-PKCζ (PKMζ) antibody (sc-216, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and mouse monoclonal anti-β-Tubulin antibody (Beyotime, Beijing, China).

### Immunofluorescence assay

The coverslips with cultured hippocampal neurons were incubated with the ECS at 37 °C for 30 minutes before experiments. The cultures were exposed to a transient OGD (3 minutes), and recovered in ECS at 37 °C for 20 minutes, and then were fixed with 4% paraformaldehyde for 30 minutes. After wash with PBS and blocking in 10% (w/v) FBS (Gibco), the neurons were labeled with GluA1 and PSD95 antibody (Millipore) overnight at 4 °C with 0.1 % Triton X-100 in PBS containing 10 % FBS. Then, neurons were incubated with Alexa 647 and Alexa 568 conjugated secondary antibody for visualizing GluA1 and PSD95, respectively. The coverslips were washed and fixed with ProLong Gold Antifade reagent (Life Technologies, Grand Island, NY, USA). Neurons were imaged using a Zeiss LSM710 laser scanning confocal microscope (Carl Zeiss, Jena, Germany) under identical conditions and analyzed using same parameters. The colocalization was determined by examination of the overlaid GluA1 and PSD95-stained image in 50 μm dendritic shaft (about 30 μm away from the cell body). The results were processed and analyzed by Fiji software (NIH, USA). The pearson′s R values were obtained and analyzed with ANOVA least significant difference (LSD) for statistical significance and are expressed as means ± SE.

### Nuclear staining

After 45-minute OGD exposure, cultures were fixed with 4% paraformaldehyde for 30 minutes, washed with PBS, and stained with Hoechst 33342 (0.5 mg/L) and propidium iodide (PI; 4 mg/L) for 1 hour before confocal imaging (LSM710, Zeiss). The number of PI or Hoechst positive nuclei was counted (Fiji software) to determine cell viability.

### Data analysis

Data were expressed as mean ± SEM. Within-group comparisons was determined by paired-sample *t*-test, and differences between groups were compared using independent-sample *t*-test (two populations) and ANOVA post-hoc comparisons. Differences were considered significant when $P$ was $<0.05$, and the significance for homogeneity of variance test was set at 0.1.

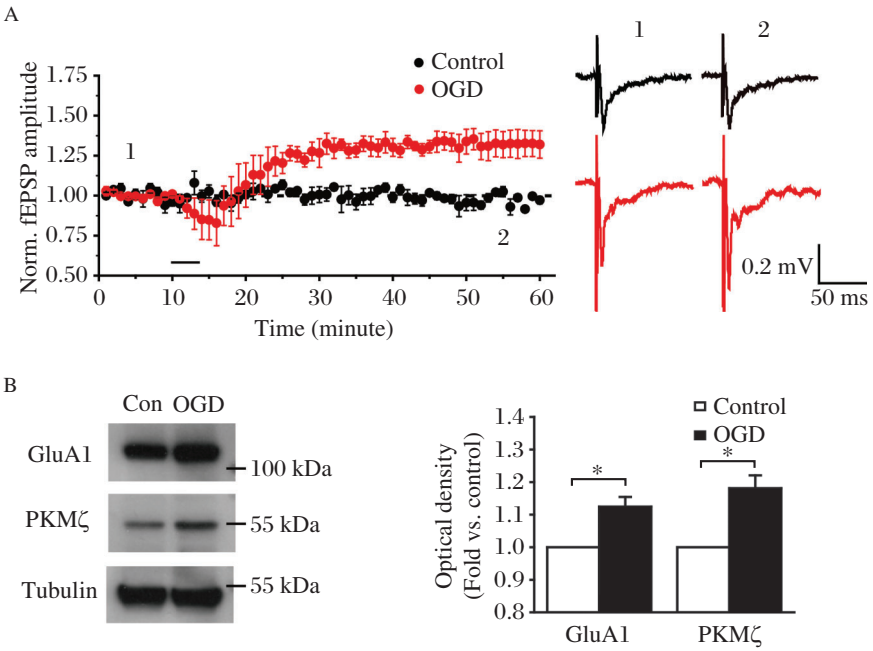


***Fig. 1.*** **Oxygen and glucose deprivation (OGD) induces post-ischemic long-term potentiation (i-LTP) and enhances GluA1 and PKMζ expression.** A: OGD induces i-LTP of field excitatory postsynaptic potentials (fEPSPs). A control was displayed to ensure stable AMPA responses during the experiment (black spots, 0.97±0.01, compared with baseline, $P>0.05$). Transient OGD (3 minutes) resulted in a significant and persistent increase of fEPSPs amplitude (red spots, 1.33±0.08, $n=5$, compared with baseline, $P<0.05$). Sample traces were obtained from the average of 10 continued sweeps at 10 minutes before (1) and 40 minutes after (2) OGD. The black line refers to the period that OGD was delivered. B: Changes of GluA1 and PKMζ with OGD were determined by Western blotting assays. There is a significant increase of GluA1/tubulin (1.12±0.03, $n=5$, $^*P<0.05$) and PKMζ/tubulin (1.18±0.04, $n=5$, $^*P<0.05$).

## Results

### I–LTP and the increases of GluA1 and PKMζ expression induced by OGD

To evaluate the pathological plasticity induced by OGD, we recorded the extracellular field potential in acute hippocampal slices. After perfusion of slices with OGD solution for 3 minutes, a persistent increase of fEPSP amplitude (***Fig. 1A***; 1.33±0.08) was recorded. According to previous studies, the augmentation of neurotransmission was mediated by the postsynaptic expression of GluA2-lacking CP-AMPARs mainly[23]. Therefore, GluA1 should be the primary AMPAR subunit which inserts into the postsynaptic membrane after OGD. Using Western blotting assay of the hippocampus tissue, we detected increased postsynaptic GluA1 expression (***Fig. 1B***; 1.12±0.03) 30 minutes after OGD. Interestingly, we found a paralleled enhancement in PKMζ expression at the same time after OGD (***Fig. 1B***; 1.18±0.04). Thus, we hypothesized that the enhancement in PKMζ expression may correlate with enhancement in GluA1 and possibly play a role in i-LTP.

### The inhibition of i–LTP by *PKMζ* knockdown

To investigate the possible role of PKMζ in i-LTP, we specifically knockdown *PKMζs* by delivering lentivirus-packed PKMζ-shRNA into hippocampal slices. Green fluorescent protein (GFP) was constructed with shRNA to ensure the successful expression of shRNA. As shown in ***Fig. 2A*** and ***Fig. 3A***, seven days (for intracranial injection) or three days (for transfetcion of cultured neurons) after lentivirus infection, GFP was expressed efficiently in both acute hippocampal slices and cultured neurons. Western blotting assays showed that the expression of PKMζ was knocked down by the shRNA efficiently (***Fig. 2B***).

After the injection of lentiviruses expressing PKMζ-shRNA or scr-shRNA into bilateral dorsal hippocampus, acute hippocampus slices were prepared and fEPSP recording was performed in the hippocampal CA1 cells with green fluorescence. As shown in ***Fig. 2C and 2D***, after transient OGD exposure, the fEPSP amplitude was increased in naive slices (1.33±0.08) and scr-shRNA transfected slices (1.35±0.07). There was no significant difference between the two groups. However, i-LTP was suppressed in

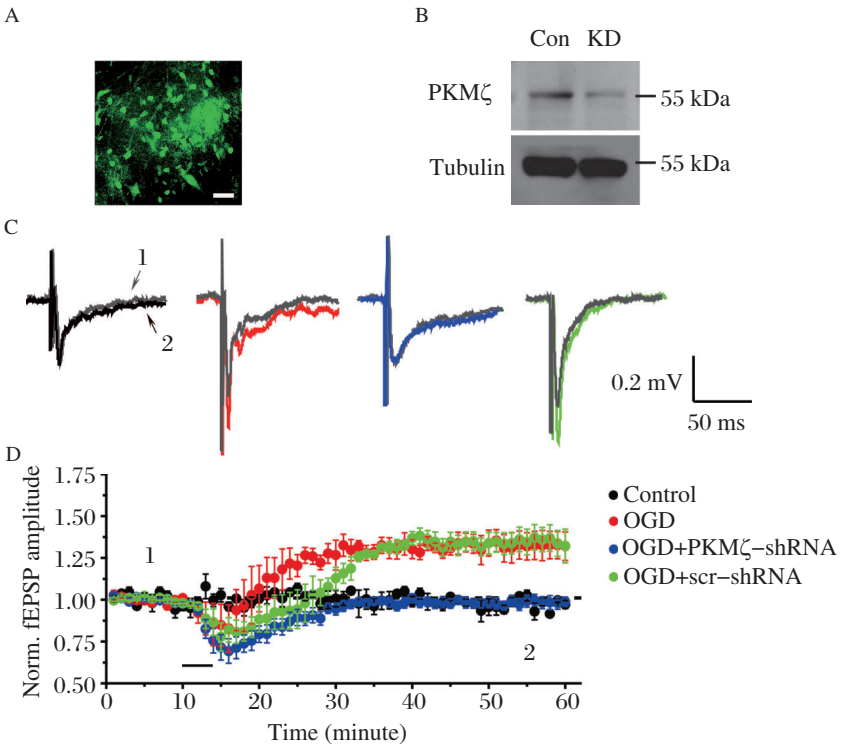


***Fig. 2. PKMζ* knockdown inhibits i-LTP expression in acute hippocampal slices.** (A) Images showing EGFP expression in dorsal hippocampal slices prepared from PKMζ-shRNA injected hemispheres following unilateral injections (4 μL) of lentivirus co-expressing PKCλ-shRNA and EGFP. Scale bar, 20 μm. (B) Confirmation of *PKMζ* knockdown. There is a reduction of PKMζ level in preparations from the dorsal hippocampus. (C) Sample traces showing fEPSPs recorded before and after OGD in transfected or non-transfected slices. (D) *PKMζ* knockdown impairs i-LTP expression (blue spot, $0.98\pm0.03$, $n=8$, compare with baseline, $P>0.05$; compared with un-injected slices, $P<0.05$). As a control, OGD-induced i-LTP was normal in slices transfected with scr-shRNA (green spot, $1.35\pm0.07$, $n=5$, compared with un-injected slices, $P>0.05$; compared with PKMζ KD, $P<0.05$). The control and OGD are borrowed from data in ***Fig. 1*** for comparison.

*PKMζ* knockdown preparations ($0.98\pm0.03$). At the same time, the effect of *PKMζ* knockdown on i-LTP was also tested in primary cultured neurons. During OGD solution perfusing, whole-cell recording of mEPSC was performed on the cultured hippocampal neurons transfected with PKMζ-shRNA or not. As shown in ***Fig. 3B and 3C***, consistent with the performance in slices, OGD treatment increased the mEPSC amplitude in control samples ($1.22\pm0.04$), but did not significantly alter the amplitude in transfected neurons ($0.94\pm0.03$). There was still obvious augmentation of mEPSC on the scr-shRNA transfecting neurons treated by OGD solution ($1.20\pm0.02$). The above data showed that *PKMζ* knockdown suppressed i-LTP both in acute slices and cultured neurons. Therefore, PKMζ was found to participate in OGD-induced synaptic plasticity.

## The involvement of PKMζ in postsynaptic expression of GluA1 during i–LTP

In physiological synaptic plasticity, LTP is mostly caused by AMPAR insertion into the postsynaptic membrane[24]. Substantial evidence supports a role for PKMζ in this process. PKMζ affects maintenance of LTP mainly by regulating the surface trafficking of AMPARs[25]. To assess the synaptic targeting of GluA1 under OGD and *PKMζ* knockdown, immunofluorescence assay was employed on cultured hippocampal neurons. The expression of GluA1 in postsynaptic sites was determined by quantifying the degree of colocalization of GluA1 and PSD95 (a postsynaptic marker). The confocal fluorescent images are shown in ***Fig. 4A***. An increased colocalization between GluA1 and PSD95 was observed, indicating that GluA1 was targeted to postsynaptic sites after OGD (***Fig. 4B***), which was aborted in neurons with *PKMζ* knockdown. No significant difference was observed between the control and the group transfected with scr-shRNA. These data suggested that OGD-induced i-LTP is mediated by increased expression of AMPARs at postsynaptic sites and PKMζ is involved in the regulation of AMPAR localization after OGD. We deduced that PKMζ plays a role in i-LTP through regulating postsynaptic expression of AMPARs.

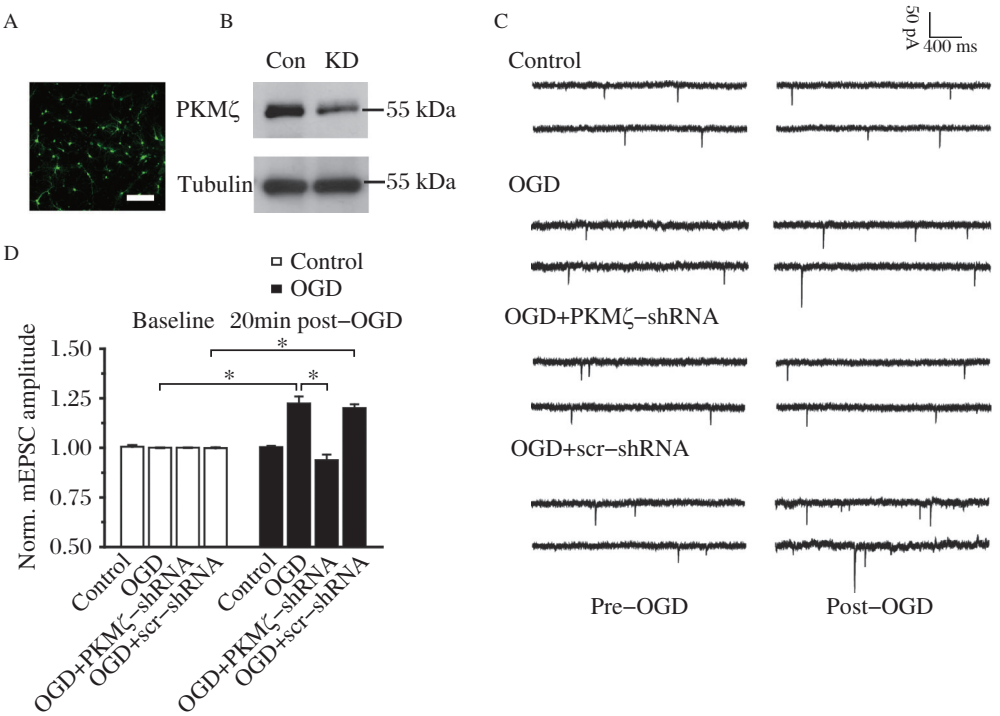


***Fig. 3. PKMζ* knockdown inhibits i-LTP expression in cultured hippocampal neurons.** A: Images showing EGFP expression in cultured hippocampal cells prepared from PKMζ-shRNA transfected with lentivirus co-expressing PKC*λ*-shRNA and EGFP. Scale bar, 50 μm. B: Confirmation of PKMζ knockdown in cultured neurons. PKMζ level is decreased. C: Sample traces before and after OGD treatment in transfected or non-transfected neurons. D: Summary of the data from similar experiments in B. OGD treatment could induce a significant increase of the mEPSP amplitude ($1.22\pm0.04$, $n=5$, $^*P<0.05$). PKMζ knockdown prevented the OGD-induced enhancement of mEPSP amplitude ($0.94\pm0.03$, $n=5$, $^*P<0.05$). The potentiation was normal in the neuron transfected with scr-shRNA. ($1.20\pm0.02$, $n=5$, $^*P<0.05$).

## Reducing neuronal death by PKMζ knockdown

The data above had revealed that PKMζ regulated the postsynaptic expression of GluA1 during i-LTP. GluA1 was thought to be the main fraction of CP-AMPARs, which mediate calcium entry and excitotoxicity in ischemic stroke[8-9]. To further investigate the functional relevance of PKMζ during OGD, cultured neurons were transfected with PKMζ-shRNA, and were subjected to OGD for 45 minutes. Then, cell death was analyzed by nuclear staining. The number of PI or Hoechst positive nuclei was counted to determine cell viability. The confocal images and statistical results are shown in ***Fig. 5***. There was a significant reduction in cell death in PKMζ-shRNA transfected samples ($0.16\pm0.01$) compared with the OGD group ($0.45\pm0.02$). Cell death was still apparent in scr-sRNA transfected neurons ($0.49\pm0.01$). The results indicated that *PKM*ζ knockdown prevents cellular damage induced by OGD. The disturbance of postsynaptic GluA1 expression by PKMζ deficiency may mediate this process.

## Discussion

LTP is observed during many physiological and pathological processes[26-27]. PKMζ is well known as a key regulator in the maintenance of physiological LTP. The presence and distribution of PKMζ in the brain and the function of PKMζ in memory have been extensively studied in recently years[13,28-30]. It is involved in AMPAR trafficking and expression at postsynaptic sites, which is the basic molecular mechanism underlying the increase of synaptic transmission. However, the mechanism of pathological LTP is still less understood. Especially, i-LTP after ischemic stroke, which affects the lives of millions of people every year, is needed to be intensively dissected. A few studies have reported the roles of PKMζ in nerve disease, such as persistent pain[14], chronic pain[15] and cervical spinal injury[31], suggesting the possible involvement of PKMζ in i-LTP. In this study, the data showed that PKMζ was up-regulated in hippocampal CA1 regions in ischemic model. PKMζ knockdown suppressed OGD-induced i-LTP in both hippocampal slices and cultured neurons. GluA1 subunit of AMPARs was selected to be analyzed due to its primary role in the potentiation of the neurotransmission during i-LTP. Indeed, the postsynaptic expression of GluA1 was attenuated by PKMζ knockdown. PICK1 is a PDZ domain-containing protein, which can bind to AMPARs *via* the PDZ domain[32] and regulate CP-AMPAR plasticity[33,34]. It was reported that the

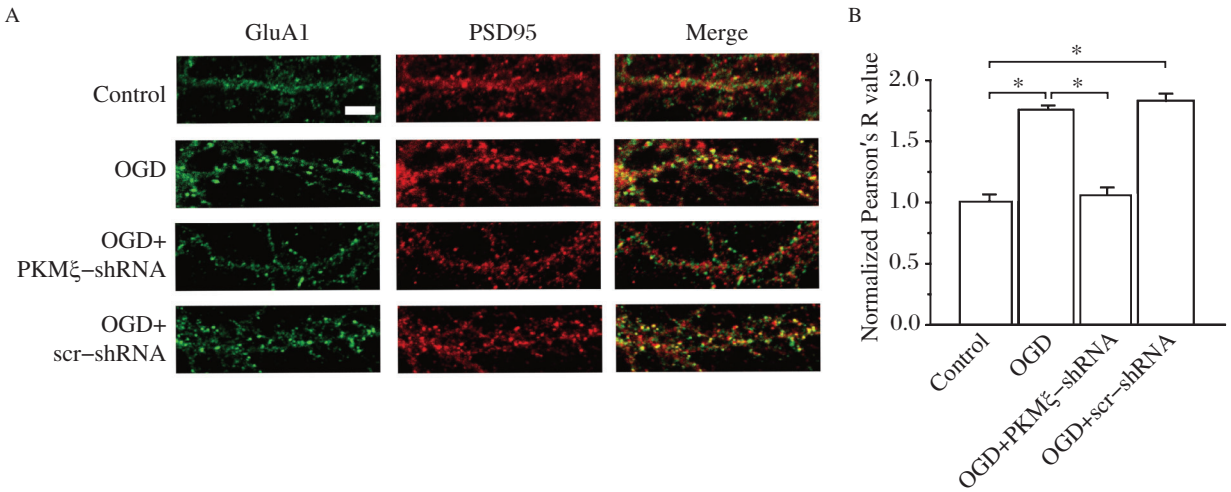


***Fig. 4.* PKMζ plays a role in the regulation of postsynaptic expression of GluA1 during i-LTP.** A: Higher magnification images of dendritic GluA1 (green) and PSD95 (red) under control and various treatments are shown. Scale bar, 2 μm. B: Quantification of colocalized GluA1 and PSD95 was represented by normalized Pearson's R value. After OGD, the colocalization of GluA1 and PSD95 was increased ($1.76\pm0.04$, $n=16$, $^*1.01\pm0.06$, $n=16$, $^*P<0.05$). *PKMζ* knockdown suppressed postsynaptic expression of GluA1 ($1.06\pm0.07$, $n=16$, $^*P<0.05$). There is no significant difference between the scr-shRNA transfected group ($1.83\pm0.06$, $n=16$, $^*P<0.05$) and the OGD group.

increase of GluA2-lacking AMPARs at synapses during OGD could be inhibited by disrupting the interactions of PICK1 PDZ domain. It was thought that this mechanism was involved in OGD-induced cell death[35]. Moreover, the C-terminal of PKMζ contains a PDZ-binding sequence that interacts with PICK1[36]. Therefore, PKMζ may regulate AMPAR trafficking in i-LTP via PICK1. Like other PKC isoforms, PKMζ is a serine and threonine kinase. It is possible that PKMζ regulates AMPAR expression by facilitating the phosphorylation of AMPAR, such as at S831 at GluA1 or S880 at GluA2. The details involved in the regulation of AMPARs by PKMζ will be further studied in the future. Furthermore, the switch from GluA2-containing AMPARs to CP-AMPARs is a main cause of ischemic injury[8-9]. GluA1-containing AMPARs mediate the main excitotoxicity during hippocampal ischemia. Therefore, it was hypothesized that PKMζ was participated in the ischemic damage by facilitating GluA1 expression at postsynaptic sites. Indeed, using OGD and cell viability test, *PKMζ* knockdown can prevent cell death.

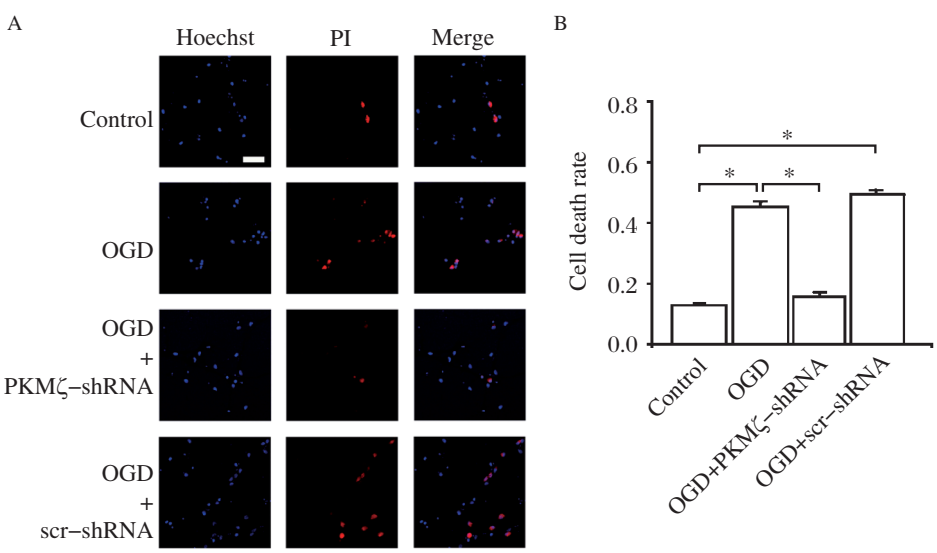


***Fig. 5.* Reduced neuronal death in *PKMζ* knockdown cultures.** A: Representative confocal images of neurons under different conditions. Cultured hippocampal neurons were exposed to 45 minutes of OGD, followed by staining with Hoechst 33258 and propidium iodide (PI). Scale bar, 50 μm. B: Summary of the data from experiments. After OGD, cell death was significantly increased ($0.45\pm0.02$, $n=6$, *compared with control $0.13\pm0.01$, $n=6$, $P<0.05$). But cell death was prevented in neurons transfected with PKMζ-shRNA ($0.16\pm0.01$, $n=6$, *compared with OGD, $P<0.05$). There is no significant change in neuronal death in the scr-shRNA group ($0.49\pm0.01$, $n=6$, *compared with control, $P<0.05$; compared with OGD, $P>0.05$).

In fact, other PKC family proteins in ischemia and hypoxia are studied widely. PKCs were reported to be altered during these processes[37-38]. PKC activity was significantly affected during the very early phase of cerebral ischemia[39-40]. As it is well known, in response to diverse signal pathways, different PKC isoforms can play multiple roles in different physiological processes and functions[37]. It is possible that all PKCs are participated in ischemia in different processes. Like in the physiological LTP, PKMζ might mainly take its role in the later phase of ischemia, but not in the early phase. All of these need to be further investigated.

In conclusion, we have shown that PKMζ plays an important role in i-LTP by regulating postsynaptic expression of AMPARs, just like its contribution to the maintenance of physiological LTP. Furthermore, PKMζ is involved in ischemic damage by facilitating GluA1 expression at postsynaptic sites. The results are helpful to understand molecular mechanisms underlying cellular damage after ischemic stroke and provide a new clue to further study in clinical research of ischemic stroke.

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