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Sequential expression of cyclooxygenase-2, glutamate receptor-2, and platelet activating factor receptor in rat hippocampal neurons after fluid percussion injury

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Abstract

Traumatic brain injury causes gene expression changes in different brain regions. Occurrence and development of traumatic brain injury are closely related, involving expression of three factors, namely cyclooxygenase-2, glutamate receptor-2, and platelet activating factor receptor. However, little is known about the correlation of these three factors and brain neuronal injury. In this study, primary cultured rat hippocampal neurons were subjected to fluid percussion injury according to Scott's method, with some modifications. RT-PCR and semi-quantitative immunocytochemical staining was used to measure the expression levels of cyclooxygenase-2, glutamate receptor-2, and platelet activating factor receptor. Our results found that cyclooxygenase-2 expression were firstly increased post-injury, and then decreased. Both mRNA and protein expression levels reached peaks at 8 and 12 hours post-injury, respectively. Similar sequential changes in glutamate receptor 2 were observed, with highest levels mRNA and protein expression at 8 and 12 hours post-injury respectively. On the contrary, the expressions of platelet activating factor receptor were firstly decreased post-injury, and then increased. Both mRNA and protein expression levels reached the lowest levels at 8 and 12 hours post-injury, respectively. Totally, our findings suggest that these three factors are involved in occurrence and development of hippocampal neuronal injury.

Key Words: *nerve regeneration; brain injury; platelet activating factor; cyclooxygenase-2; RT-PCR; immunocytochemistry; hippocampus; platelet activating factor receptor; glutamate receptor 2; NSFC grant; neural regeneration*

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Introduction

Brain injury induces a sequence of gene expression that includes cyclooxygenase-2 (COX-2), glutamate receptor 2 (GluR2), and platelet activating factor receptor (PAFR). These three factors are closely related and play a synergic role (Mukherjee et al., 1999). To date, few investigations have focused directly on the correlation between these three factors and hippocampal neurons. Platelet activating factor is an important active mediator in brain injury pathology, triggering and directly involved in pathological impairment of brain tissue. Increasing evidence suggests that the pathophysiological effects of platelet activating factor are mediated by intracellular signal transduction after binding to PAFR (Pulliam et al., 1998). In addition, platelet activating factor production is accompanied simultaneously by arachidonic acid and its metabolites, especially generation and release of epoxide-dependent thromboxane A2 and prostaglandin I2. Under normal physiological conditions, thromboxane A2 and prostaglandin I2 are well balanced, allowing maintenance of normal vascular tone and blood vessel patency. Disordered balance between thromboxane A2 and prostaglandin I2 causes vascular spasms and occlusion (Bulger et al., 2000). Platelet activating factor also promotes glutamate excitotoxicity (Aihara et al., 2000).

Growing experimental evidence shows that brain injury leads to increased COX-2 mRNA, protein, and reaction products, and COX-2 metabolites may aggravate brain injury and brain neuronal damage *via* the vasculature (Busija et al., 1996; Domoki et al., 1999). These findings highlight the contribution of COX-2 to brain injury. GluR2 mediates sodium, water, and chloride influx after brain injury, leading to neuronal excitotoxicity and deteriorating brain damage (Iversen et al., 1994; Pellegrini-Giampietro et al., 1994; Essin et al., 2002).

Overall, these studies suggest that PAFR, GluR2, and COX-2 play crucial roles in brain injury pathogenesis, and potentially interact with one another to promote development of brain injury. In this study, we used primary cultured hippocampal neurons to establish a fluid percussion injury model, and investigated sequential changes in PAFR, COX-2, and GluR2 mRNA and protein expression levels following neuronal injury.

Materials and Methods

Animals

Forty healthy Sprague-Dawley rats within 24 hours of birth (weight 5–7 g) were provided by the Experimental Animal Center of the Academy of Military Medical Sciences, China (license No. SCXK (Army) 2006-001).

Primary cultures of rat hippocampal neurons

For cell growth observation, coverslips were divided into four small pieces and placed in petri dishes (Falcon 60 mm), with three coverslips per dish. To facilitate cell growth, 24 hours before neuronal harvesting, petri dishes were covered with 10 μg/mL polylysine and incubated overnight at 37°C. The next day, petri dishes were rinsed three times with D-Hank's solution. Sprague-Dawley rats were immersed in 75% ethanol and disinfected. Brain tissue was harvested by decapitation, and the hippocampus isolated and placed in D-Hank's solution. Hippocampal tissue was cut into pieces and repeatedly pipetted into tiny blocks before filtering through a 200 mesh filter to prepare cell suspensions. One drop of cell suspension was mixed with one drop of 0.04% trypan blue (Gibco, Grand Island, NY, USA) and counted by light microscopy. The cell suspension was diluted using DMEM-F12 medium to a concentration of $2–4 \times 10^5$ cells/mL, and then 10% fetal bovine serum (Institute of Hematology, Chinese Academy of Medical Sciences, Tianjin, China) and 1% penicillin-streptomycin added. Cells were incubated at 37°C in a 5% CO_2 incubator (Forma 3111; Thermo Scientific, CA, USA) for 48 hours. After removing fetal bovine serum, culture medium with 5% N_2 additives (Gibco), 2.5% glucose and 1% penicillin-streptomycin was added. Half the culture medium was changed every 3–4 days, and cells were cultured for 7–10 days.

Grouping and intervention

Experimental rats were randomly divided into control and five injury (4, 8, 12, 24, and 48 hours after traumatic brain injury) groups. Hippocampal neurons in the control group were cultured normally, without fluid percussion injury. The injury groups were subjected to fluid percussion injury and RT-PCR or immunocytochemistry performed at corresponding time points post-injury.

Fluid percussion injury model established using a modified Scott's method

Based on a previously described method (Shepard et al., 1991), we established a fluid percussion injury model in nerve cells using a modified injury device, which consists of a stainless steel cell injury chamber, a plexiglass cylinder, a pressure transducer, a pendulum frame, and an oscilloscope (**Figure 1**). The plexiglass cylinder connects to the piston at one end, and to the injury chamber and pressure transducer at the other end. The cylinder is filled with 37°C saline during the injury process. When the pendulum hammer is lifted to a certain height, the falling weight hits the piston and pushes the fluid forward, leading to damage to cells cultured in dishes. This model was confirmed a success in preliminary studies (He et al., 2010). In brief, after discarding culture medium, cells in petri dishes were digested with 75% ethanol, placed in the injury chamber containing D-Hank's solution, and tightly covered. Next, the pendulum hammer was lifted to a 25° angle, defining an impact force of 0.1 MPa. Subsequently, the culture medium was replenished and cells cultured in an incubator for RT-PCR or immunohistochemistry analysis.

Immunocytochemistry using the SP method

Neuronal identification

On day 7 after culture, coverslips were removed from petri dishes, placed into 24-well plates, fixed in 4% paraformaldehyde for 4 hours, and rinsed 3 times for 5 minutes with 0.01 mol/L PBS. Coverslips were then incubated with 3% H_2O_2 at room temperature for 20 minutes to eliminate endogenous peroxidases, washed with PBS, and incubated with 0.3% Triton X-100 at 37°C for 30 minutes. Subsequently, cells were rinsed with PBS, blocked with 10% horse serum at room temperature for 30 minutes, and then incubated overnight at 37°C with mouse anti-rat microtubule-associated protein-2 (1:100) and glial fibrillary acidic protein (1:400) monoclonal antibodies (Fuzhou Maxin Biotechnology Company, Fuzhou, Fujian Province, China). After recovery at room temperature for 15–20 minutes and washing with 0.01 mol/L PBS, coverslips were incubated with biotinylated horse anti-mouse IgG (1:200; Beijing Zhongshan Biotechnology Co., Ltd., Beijing, China) at 37°C for 30 minutes, then incubated with SP complex (1:200) for 30 minutes, and developed with DAB for 2–3 minutes. Between steps, cells were rinsed 3 times for 5 minutes with 0.01 mol/L PBS. After terminating DAB coloration with PBS, coverslips were subjected to ethanol dehydration, xylene transparency, and mounting. PBS was used as the primary antibody for negative controls. The number of microtubule-associated protein-2- and glial fibrillary acidic protein-positive cells was calculated, and the percentage of positive cells to total cells was measured as the purification rate of neuronal cells.

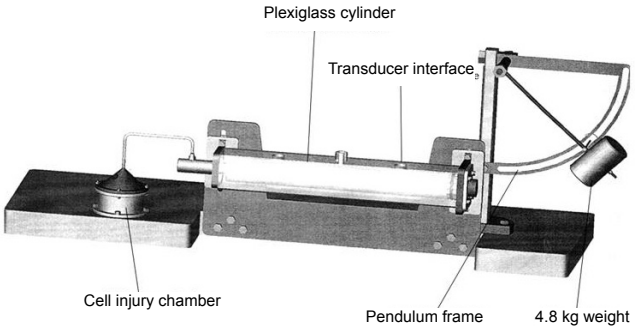

Figure 1 Scott's model of fluid pressure injury.
The fundamental principle is to culture cells with fluid at a certain impact velocity. We modified the injury device proposed by Scott et al. (1991), which consists of a stainless steel cell injury chamber, a plexiglass cylinder, a pressure transducer, a pendulum frame, and an oscilloscope.

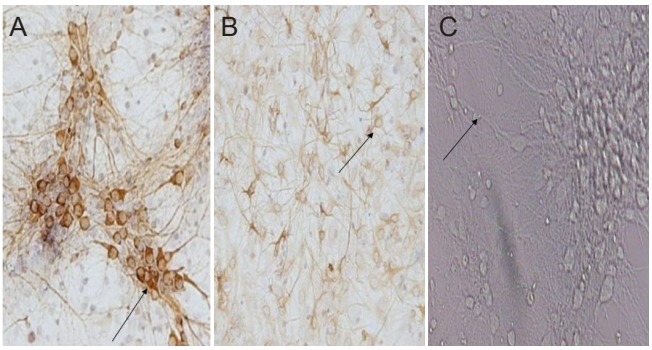

Figure 2 Morphology of rat hippocampal neurons at 7 days of culture.
Neuronal purity was determined using immunohistochemical staining. (A) Hippocampal neurons (arrow) labeled by MAP2 and imaged using an upright light microscope. (B) Hippocampal astrocytes (arrow) labeled by GFAP and imaged using an upright light microscope. (A, B) × 400. (C) Hippocampal neurons (arrow) imaged using an inverted light microscope (× 200).

Immunocytochemistry detection of PAFR, COX-2, and GluR2
Cells were blocked with 10% rabbit serum at room temperature and incubated overnight at 37°C with the following antibodies: goat anti-PAFR, COX-2, and GluR2 polyclonal antibodies (1:200; Santa Cruz Biotechnology, Santa Cruz, CA, USA), followed by biotinylated goat anti-goat IgG (1:200; Beijing Zhongshan Biotechnology Co., Ltd.) for 30 minutes at 37°C. The rest of the procedure is the same as for microtubule-associated protein-2 and glial fibrillary acidic protein immunocytochemistry.

Quantitative image analysis
Images were quantitatively analyzed using the Mias2000 analysis system (Sichuan University, Chengdu, Sichuan Province, China). Images of hippocampal neurons were collected using a light microscope (Olympus BX-51, Tokyo, Japan) under the same light intensity at 400 × magnification. Immunoreactive areas of 100 cells (μm^2) were calculated from each slide.

Table 1 PCR primers

Gene	Sequence (5'–3')	Product size (bp)
COX-2	Upstream: CTG TAT CCC GCC CTG CTG GT Downstream: GAG GCA CTT GCG TTG ATG GT	287
GluR2	Upstream: CCT CAG AAG TCC AAG CCA GGA GTG Downstream: CAG GAA GGC AGC TAA GTT AGC CG	353
PAFR	Upstream: CAC TTA TAA CCG CTA CCA GGC AG Downstream: AAG ACA GTG CAG ACC ATC CAC AG	381
β-Actin	Upstream: GAT CTT GAT CTT CAT GGT GCT AGG Downstream: TTG TAA CCA ACT GGG ACG ATA TGG	764

Table 2 Relative expression (/β-actin) of PAFR, COX-2, and GluR2 gene in primary cultured rat hippocampal neurons after injury

Group	PAFR	COX-2	GluR2
Control	0.19±0.03	0.26±0.05	0.63±0.12
4 h post-injury	0.16±0.02	0.30±0.09	0.49±0.10
8 h post-injury	0.13±0.02[a]	0.46±0.16[a]	0.34±0.06[a]
12 h post-injury	0.14±0.01[a]	0.63±0.10[a]	0.37±0.10[a]
24 h post-injury	0.16±0.03	0.30±0.06	0.45±0.12
48 h post-injury	0.18±0.04	0.26±0.04	0.54±0.18

[a]$P < 0.05$, *vs.* control group. Data are expressed as mean ± SD of six rats per group (one-way analysis of variance and least significant difference test). Relative expression of target mRNA was calculated using absorbance ratio of target/β-actin mRNA. PAFR: Platelet-activating factor receptor; COX-2: cyclooxygenase-2; GluR2: glutamate receptor 2. h: hours.

RT-PCR detection

RNA extraction
Total RNA was extracted from primary cultured rat hippocampal neurons using TRIzol reagent (Sigma, St. Louis, MO, USA), and stored at –80°C.

Reverse transcription
Extracted RNA was transcribed to cDNA using a RT-PCR kit (Promega, Madison, WI, USA).

PCR amplification
PCR master mix (50 μL) consisted of 5 × buffer (10 μL), TaKaRa Taq (0.25 μL), sterile distilled water (28.75 μL), primers (**Table 1**; 0.25 μL), and cDNA template (5 μL). Amplification was performed using a PCR instrument (PT-0200; MJ Research, Waltham, MA, USA), with the following conditions: an initial denaturation at 94°C for 2 minutes, then 30 cycles of 94°C for 30 seconds, primer annealing at appropriate temperatures (PAFR, 60°C; COX-2, 55°C; and GluR2, 58°C) for 30 seconds, and extension at 72°C for 30 seconds, with a final extension at 72°C for 7 minutes.

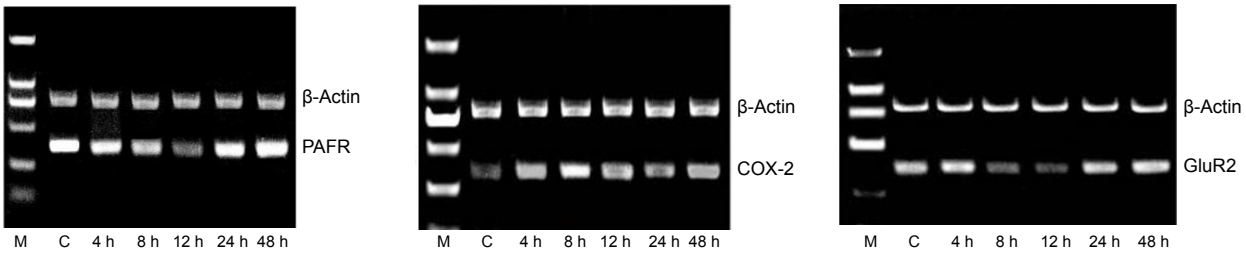

Figure 3 *PAFR* (381 bp), *COX-2* (287 bp), and *GluR2* (352 bp) mRNA expression detected by RT-PCR in hippocampal neurons at different injury time points.
M: Marker; C: control group; 4 h, 8 h, 12 h, 24 h, and 48 h: post-injury 4, 8, 12, 24, and 48 h groups; h: hours. Marker molecular weight is 2,000, 1,000, 750, 500, 250, and 100 bp from top to bottom. PAFR: Platelet-activating factor receptor; COX-2: cyclooxygenase-2; GluR2: glutamate receptor 2. β-Actin was used for normalization purposes.

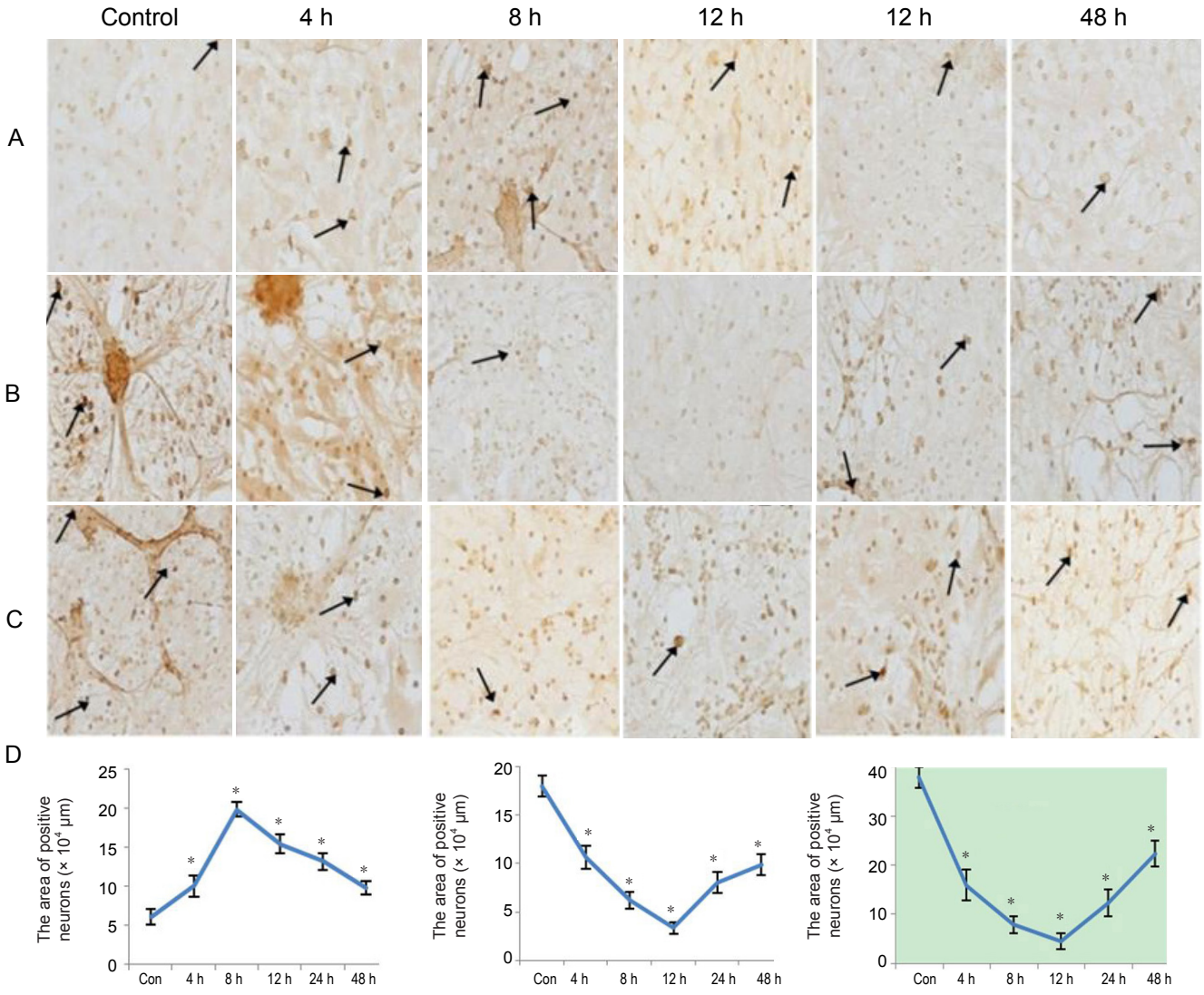

Figure 4 Immunohistochemical staining of COX-2, GluR2, and PAFR in hippocampal neurons 4–48 hours after injury.
Arrows indicate positively stained cells for COX-2 (A), GluR2 (B), and PAFR (C) (× 200). Areas of positive hippocampal neurons in the different injury groups were compared to the control group (D). *$P < 0.01$, *vs.* control group. Data are expressed as mean ± SD of six dishes of cells for each group (one-way analysis of variance and least significant difference test). COX-2: Cyclooxygenase-2; GluR2: glutamate receptor 2; PAFR: platelet-activating factor receptor; h: hours.

Agarose gel electrophoresis

PCR products (5 μL) were electrophoresed and photographed under ultraviolet light. Absorbance was measured with β-actin serving as a reference. Absorbance values of amplified bands were obtained using a gel imaging analysis system (GelPro 4.5; Media Cybernetics, Silver Spring, MD, USA). Ratios of absorbance values for PAFR, COX-2, and GluR2 amplified bands (Af) to the absorbance value for the β-actin amplified band (Ab) were calculated as the relative content of RT-PCR products.

Statistical analysis

Measurement data were expressed as mean ± SD and analyzed using SPSS 11.5 software (SPSS, Chicago, IL, USA). Differences between groups were compared using one-way analysis of variance and least significant difference tests. A $P < 0.05$ value was considered statistically significant.

Results

Morphology of hippocampal neurons

After *in vitro* culture for 2–4 hours, hippocampal neurons began to adhere and were scattered or grew in clusters. Adherent cells were round, small, and stereoscopic, with a visible halo. At 24 hours, some neurons extended processes. At 48 hours, N_2 was added to the culture medium and non-adherent cells removed. The cultured neurons became larger, with granular substances in cell bodies, a clearly visible halo around cells, and ingrowth of processes. At 3 days, the majority of neurons extended processes and adjacent cells also exhibited fiber connections. Cultured neurons in clusters began to migrate towards peripheral tissue. At 5–7 days, the cells were most vital, exhibiting strong refraction and nuclei located in the center or to one side of the cells, with the nucleus and nucleolus clearly visible. Neurites were thick and formed a network.

Identification of rat hippocampal neurons

Microtubule-associated protein-2 is a neuronal specific marker, and after 7 days of culture was used to identify neurons. Rat hippocampal neurons were microtubule-associated protein-2 positive, with positive substance stained brown and located in the cytoplasm and dendrites. No microtubule-associated protein-2 expression was found in glial cells. The percentage of positive cells (purification rate of cultured neurons) was ≤ 75%. Glial fibrillary acidic protein is an astrocyte specific marker, and was ubiquitously expressed in glial cells, with positive substance stained brownish yellow and located in the cytoskeleton. Minimal glial fibrillary acidic protein expression was found in neurons, specifically, primitive immature neurons (**Figure 2**).

COX-2, GluR2, and PAFR mRNA expression in rat hippocampal neurons

COX-2 mRNA expression

COX-2 mRNA expression was relatively low in the control group, although expression levels gradually increased along with time post-injury and reached a peak at 12 hours before decreasing. COX-2 mRNA expression in the post-injury 8 and 12 hour groups was higher than controls ($P < 0.05$; **Table 2, Figure 3**).

GluR2 and PAFR mRNA expression

GluR2 and PAFR mRNA expression was relatively high in the control group, with expression levels gradually decreasing at 4 hours post-injury, reaching a lowest level at 8 hours before increasing. GluR2 and PAFR mRNA expression in the post-injury 8 and 12 hour groups was significantly lower than in controls ($P < 0.05$; **Table 2, Figure 3**).

COX-2, GluR2, and PAFR protein expression in rat hippocampal neurons

COX-2 immunocytochemical staining results

In the control group, a small number of COX-2 positive cells were hippocampal neurons with brown positive substance located in the cytoplasm and nuclear membrane. COX-2 was strongly expressed in the nuclear membrane, but negatively expressed within nuclei. The cytoplasm was slightly stained, and twisted and spiral dendrites and axons grew in some cells. Staining was weakest in the control group and COX-2 immunoreactivity increased in the injury groups, reaching a peak at 8 hours. Neuronal swelling and rupture, cytoplasmic leakage, neurite breakage, and cell shrinkage were visible after injury. Subsequently, darkly stained cytoplasm, nuclear condensation, and increased cell debris were observed. The degree of staining was attenuated with reduced COX-2 positive cells, albeit with greater numbers than in the control group.

A small number of COX-2 immunoreactive cells were expressed in the control group, with cell numbers increasing after injury. The number of COX-2 immunoreactive cells in hippocampal neurons after injury was higher than the control group ($P < 0.01$), reaching a peak at 8 hours (**Figure 4A, D**).

GluR2 immunocytochemical staining results

GluR2 immunoreactive cells were strongly and widely expressed in the control group. Positive substance was brown and located in the cytoplasm, which showed strong expression, while no nuclear expression was found. Some neurons extended positive, short processes. In the injury groups, immunoreactive expression of neurons decreased, and GluR2 content in positive cells also decreased. At 4 hours post-injury, neuronal cell bodies were swollen and connections between neuronal processes interrupted. Subsequently, cell bodies ruptured, a large amount of cell debris were visible, and neuronal processes exhibited fracture. Residual cells began to shrink, with darkly stained cytoplasm and pyknotic nuclei. The number of GluR2 positive cells was significantly reduced at 48 hours because of neuronal death.

A large number of GluR2 immunoreactive cells were expressed in the control group, with a reduced number after injury. The number of neuronal GluR2 immunoreactive cells after injury was significantly lower than the control group ($P < 0.01$), with a lowest level at 12 hours (**Figure 4B, D**).

PAFR immunocytochemical staining results

In the control group, PAFR was widely expressed in hippo-

campal neurons, with brown positive substance located in the cytoplasm and nucleus. After injury, neuronal cell bodies were swollen, intercellular connections interrupted, and the cytoplasm irregularly and lightly stained, showing nuclear enlargement or disappearance, cell body rupture, and cell debris. Subsequently, cell shrinkage, darkly stained cytoplasm, pyknotic nuclei, and cell debris were clearly visible. The number of PAFR positive cells was reduced at 48 hours because of neuronal death.

PAFR immunoreactive cells were widely expressed in the control group, while the number reduced after injury. Expression of PAFR immunoreactive cells in hippocampal neurons after injury was lower than the control group ($P < 0.01$), with a lowest level at 12 hours post-injury (**Figure 4C, D**).

Discussion

Increasing evidence from proteomic studies of traumatic brain injury (Blain et al., 2004; Conti et al., 2004; Siman et al., 2004; Sironi et al., 2004; Ding et al., 2006; Glanzer et al., 2007; Crecelius et al., 2008) has provided novel pathways for clinical treatment, and been beneficial for improving the quality of life among patients. Preliminary studies from our research group using the SELDI-TOF MS technique, have detected protein expression profiles in serum and hippocampus of rats with closed traumatic brain injury (Shu et al., 2005; Zhan et al., 2007; Shu et al., 2010). Our results indicate that brain injury simultaneously causes alterations of protein expression profiling in serum and hippocampus. Although the serum and hippocampal protein expression profiles are different, it is still possible to detect the same protein peaks in two samples using the same chip. We speculate that during occurrence and development of brain injury, cellular protein changes can reflect serum changes. After injury, some hippocampal protein changes precede those in serum, and *vice versa*. A possible explanation for this discrepancy is destruction of the blood-brain barrier, and then increased permeability, inflammation, immune response, and oxidation reactions (Hellal et al., 2004; Nimmo et al., 2004; Grond-Ginsbach et al., 2008).

Establishment of a brain injury model allows further investigation of organ functions and related treatments (Wasinger et al., 1995; Tao et al., 2004). The cellular mechanism of brain injury has been the subject of intense investigations. In this study, we applied Scott's method, with some modifications, for producing fluid percussion injury in nerve cells, to examine changes and the underlying cellular mechanisms after moderate neuronal injury caused by a 0.2 MPa impact.

COX-2 cannot be detected in most normal tissues and its expression is induced by a variety of physiological stimuli (Subbaramaiah et al., 1997). In the adult rat brain, COX-2 is mainly distributed in the hippocampus and temporal cortex, which are related because of the high densities of excitatory amino acid receptors in both (Kulmacz et al., 1995). After traumatic brain injury caused by fluid percussion, Dash et al. (2000) found increased COX-2 protein levels in the rat hippocampus at 3 hours, which reached a peak at 24 hours. Similarly, using RNA probe hybridization studies, Strauss et al. (2000) found that hippocampal COX-2 mRNA expression reached a peak at 6 hours before decreasing after brain injury caused by local impact. In previous studies from our research group, we duplicated Marmarou's closed head injury model in rats (Xiong et al., 2013) and using RT-PCR found that hippocampal COX-2 mRNA expression begins to increase at 4 hours after brain injury, reaching a peak at 8 hours before gradually declining, but is still higher than the control group at 48 hours (Yu et al., 2008). These experimental findings suggest that COX-2 gene transcription and protein expression levels sharply increase and then gradually decrease in the rat hippocampus after fluid percussion injury.

Glutamate is the major excitatory neurotransmitter of the central nervous system. AMPA receptors mediate fast excitatory synaptic transmission, and are composed of a central hydrophilic channel surrounded by GluR1, GluR2, GluR3, and GluR4 subunits that form a dimer structure. AMPA receptor permeability to Ca^{2+} is controlled by GluR2 (Bettler et al., 1995; Seeburg et al., 1998). Once injury occurs, GluR2 is reduced, leading to formation of GluR1- or GluR3-dominant ion channels that mediate quick Ca^{2+} influx and thereby cause nerve cell damage. Using Marmarou's closed head injury model in rats, we found by RT-PCR that hippocampal GluR2 mRNA expression begins to decrease at 4 hours after brain injury, reaching a lowest level at 12 hours before gradually increasing, yet is still lower than the control group at 48 hours (Strauss et al., 2000). These experimental findings suggest that GluR2 gene transcription and protein expression levels in hippocampal neurons decrease rapidly and then gradually increase, consistent with our current results. Moreover, changes in protein expression levels occurred after changes in gene transcription.

Platelet activating factor is a membrane phospholipid metabolite. Platelet activating factor generation increases during various wounds, and nerve cells may release platelet activating factor after stimulation. Cerebral ischemia causes a sharp increase in platelet activating factor and decreased cortical PAFR gene expression levels, suggesting PAFR synthesis within the cerebral ischemic area gradually reduces, resulting in decreased receptor levels. Using a rat model of focal ischemia-reperfusion injury, Zhang et al. (1995) found that PAFR gene expression levels significantly decreased, reaching lowest levels at 48 hours for 5 days. Following cerebral ischemia, reduced PAFR mRNA levels may be a reactive negative feedback regulation (Stengel et al., 1997). The main mechanism of decreased PAFR expression is mediated by intracellular migration of PAFR, which are gradually degraded by lysosomes. Using Marmarou's closed head injury model, we found by RT-PCR that hippocampal PAFR mRNA expression begins to decrease at 4 hours post-injury, reaching a lowest value at 8 hours before gradually increasing, but is still lower than normal levels at 48 hours (Zhang et al., 1995). The present study shows that PAFR mRNA expression is high in the control group, but sharply decreases and then gradually increases after injury.

Platelet activating factor transduced by its receptor can ac-

tivate cytosolic PLA2 in nerve cells, and cytosolic PLA2 is the mediator for synthesis between platelet activating factor and arachidonic acid, with the metabolism of all three closely related (Farooqui et al., 1997). arachidonic acid eicosanoids are generated during platelet activating factor reproduction and synthesis, which in turn produce prostaglandins, thromboxanes, leukotrienes, and other active substances. These active substances are important factors for brain injury deterioration (Shindon et al., 2000). In nerve cells, platelet activating factor binds to high-affinity sites on intracellular receptors, activating the FOS/JUN/AP-1 transcription signaling system and the COX-2 gene, thereby promoting arachidonic acid metabolism (Musto et al., 2011). COX-2 can enhance the toxic effects of excitatory amino acids, and prostaglandin E2, its reaction product, is responsible for NMDA receptor-mediated neurotoxic effects that cause brain damage, potentially by increasing microglial Ca^{2+} influx and promoting glutamate release (Li et al., 2008). Following brain injury, there is enhanced synaptic activity, GluR2 activation, increased COX-2 expression, and increased reaction products, suggesting that glutamate promotes COX-2 expression. In primary cultured neurons and microglia cells, glutamate induces platelet activating factor synthesis and release, enhancing chemotaxis of microglia cells *via* PAFR, and leading to brain injury deterioration (Aihara et al., 2000). Platelet activating factor also functions to promote presynaptic and postsynaptic glutamate release, and facilitates glutamate-mediated neurotransmission. The latter acts on postsynaptic NMDA and AMPA/kainate receptors, thus increasing cytosolic free Ca^{2+} and activating the cytosolic PLA2 system, which in turn generates platelet activating factor and arachidonic acid.

In summary, PAFR, COX-2, and GluR2 jointly promote occurrence and development of hippocampal neuronal injury following brain injury.

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Conflicts of interest: *None declared.*

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