The role of protein kinase C in the opening of blood–brain barrier induced by electromagnetic pulse

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The aim of this study was to determine the role of protein kinase C signaling in electromagnetic pulse (EMP)-induced blood–brain barrier (BBB) permeability change in rats. The protein level of total PKC and two PKC isoforms (PKC-α, and PKC-β II) were determined in brain cerebral cortex microvessels by Western blot after exposing rats to EMP at 200 kV/m for 200 pulses with 1 Hz repetition rate. It was found that the protein level of PKC and PKC-β II (but not PKC-α) in cerebral cortex microvessels increased significantly at 0.5 h and 1 h after EMP exposure compared with sham-exposed animals and then recovered at 3 h. A specific PKC antagonist (H7) almost blocked EMP-induced BBB permeability change. EMP-induced BBB tight junction protein ZO-1 translocation was also inhibited. Our data indicated that PKC signaling was involved in EMP-induced BBB permeability change and ZO-1 translocation in rat.

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1. Introduction

The blood–brain barrier (BBB) contributes to the brain homeostasis by controlling the entry of endogenous and exogenous compounds into the brain, and maintains optimal conditions for neuronal and glial functions (Wolburg and Lippoldt, 2002). This unique selective barrier is mainly supported by tight junctions (TJs) between cerebral endothelial cells (Hawkins and Davis, 2005). Till now, several tight junction-associated protein components have been identified in cerebral endothelial cell TJs, such as occludin (Furuse et al., 1993), claudin-1, claudin-5 (Liebner et al., 2000b) and submembranous components ZO-1 (Stevenson et al., 1986), ZO-2 (Jesaitis and Goodenough, 1994) and ZO-3/p130 (Balda et al., 1993).

Animal and human studies have shown that electromagnetic fields (EMFs) are capable of inducing BBB disruption. In 1977, it was first reported that a higher power microwave exposure yielded p-mannitol leakage from BBB, and pulse wave was more effective in the BBB permeability enhancement than continuous wave (Oscar and Hawkins, 1977). Williams et al. (1984a) reported an increased intensity of sodium fluorescein (a tracer of BBB permeability) in brain tissue after exposing rats to 2450-MHz microwave for 30 min. In an in vitro study, two-fold increase in BBB permeability was observed after exposure cells to 1.8 GHz EMF over 4 days (Schirmacher et al., 2000). In another study, temporary alteration in BBB permeability was found after exposure rats to 2450-MHz microwave for 2 h (Williams et al., 1984b). Previously, we reported that exposure to electromagnetic pulse (EMP) led to an increase in BBB permeability in rats (Ding et al., 2009a), and the peak of BBB opening was found at 3 h, meanwhile, the altered distribution of TJ protein ZO-1 was found at 3 h after 200 kV/m 200 pulses EMP exposure (Qiu et al., 2009). EMP used in our study is a short high-voltage pulse with an extremely fast rising time and a broad bandwidth. This kind of signal can be generated by nuclear bomb explosion. EMP signals also exist in certain occupational conditions, for example, Pulse Power Technology Lab, in which the strong electrical field apparatus such as high pressure gas switch and Tesla transformer generator can generate EMP. The unusual properties of

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EMP have raised concerns about their biological effects and possible health hazard to humans, especially to some military personnel, some workers or researchers who work with or can be exposed to this kind of electromagnetic field in their working environment. However, the biological effects of EMP exposures remain unclear (Merritt et al., 1995; Adair, 1995).

It has been suggested that the signaling pathway of protein kinase C (PKC), cyclic AMP (cAMP)-dependent protein kinase (PKA), cyclic GMP (cGMP)-dependent protein kinase (PKG), mitogen-activated protein kinase (MAPK), and nonreceptor protein tyrosine kinases are involved in the modulation of BBB structure and function (Yuan, 2003). PKC, a family of serine/threonine kinases, plays a key role in diverse intracellular signaling processes, including regulating the BBB TJ assembly and permeability (Karczewski and Groot, 2000). For example, decreased transendothelial electrical resistance (TER) and increased paracellular flux were observed in cells exposed to the PKC agonist (12-O-tetradecanoylphorbol-13-acetate) (Clarke et al., 2000). PKC family is categorized into three subclasses: Ca2+/phospholipid-dependent conventional PKC-α, β, and γ, Ca2+-independent novel PKC-δ, ε, η, and ζ; and phospholipid-independent atypical PKC-ζ and λ (Tanaka and Nishizuka, 1994). It was shown that PKC-α, β, γ, δ, ε, η, ζ, and ζ isozymes were expressed in rat brain tissue; and a similar expression pattern was seen in freshly purified microvessels, but the PKC-γ could not be detected (Krizbai et al., 1995). Some evidence supported that PKC could regulate the phosphorylation and cellular localization of occludin (Anna et al., 2001) and translocation of ZO-1 (Chen et al., 2002). The aim of this study was to investigate whether PKC signaling contributed to EMP-induced BBB permeability change.

2. Materials and methods

2.1. Materials

SDS, acrylamide, and bisacrylamide were purchased from Sigma. Goat polyclonal antibody directed to albumin was obtained from Bethyl Lab (USA). Rabbit polyclonal antibody directed to ZO-1 (BA1335), and rabbit polyclonal antibody directed to PKC-β (BA1358) were purchased from Boster Biological Technology, Ltd (Wuhan, China). As a loading control for Western blot, mouse monoclonal antibody directed to β-actin was purchased from ZSCB-BIO (Beijing, China). Horseradish peroxidase-linked anti-rabbit secondary antibody (ZB2301), anti-goat secondary antibody (ZB2306) and anti-mouse secondary antibody (Sy-5507) were purchased from ZSCB-BIO (Beijing, China). Molecular weight standards for Western blot were obtained from Fermentas (SM1841, USA). PKC inhibitor H7 (1S-chloronaphthalene-1-sulfonyl)-1H-hexahydro-1,4-diazepine) (H891) was purchased from Sigma (USA).

2.2. EMP exposure apparatus

An all-solid-state nanosecond generator has been developed and tested in Northwest Institute of Nuclear Technology in Xi’an, China, which is described elsewhere (Ding et al., 2009b). Briefly, the generator is composed of three relatively independent units: resonant charging unit, magnetic pulse compression unit, and semiconductor opening switch (SOS) unit. The resonant charging unit performs regulated primary pulse from power mains, and then the magnetic pulse compression unit, including magnetic saturation pulse transformers and magnetic switches, increases the pulse voltage and compresses the pulse width. Because of the effect of forward and reverse pumping current, SOS cuts off the current immediately. The energy stored in the inductor is eventually transferred to the load; meanwhile, high voltage and short pulse output is realized. The electric filed in exposure area within 30 cm × 30 cm × 30 cm is uniform. 200 kV/m EMP pulses with 3.5 ns rising time, 14 ns pulse width, and 1 Hz repetitive rate were used in this experiment. The output waveform from EMP generator was shown in Fig. 1.

2.3. Animals

The experimental protocol used in this study was approved by the Ethics Committee for Animal Experimentation of the Fourth Military Medical University and was conducted according to the Guidelines for Animal Experimentation of the Fourth Military Medical University (Xi’an, Shaanxi, China). Male Sprague–Dawley rats weighing 200–250 g were obtained from Animal Center of the Fourth Military Medical University (Xi’an, China). The animals were housed in stainless-steel cages in a temperature-controlled, 12/12 light/dark room, and allowed to have free access to semi-purified rat chow as well as pre-prepared drinking water. The animals were sham or whole-body exposed to EMP at 200 kV/m for 200 pulses, the repetition rate was 1 Hz. During exposure, the rats were awake and not restrained in the exposure chamber. The temperature measurements were done immediately before and after EMP exposure. The exposure produced a rise in rat rectal temperature less than 0.2 °C.

2.4. Experimental groups

For determining the protein level of total PKC, PKC-α and PKC-βII after EMP exposure, 30 rats were divided randomly into 5 groups (n = 6) as follows. One sham group and four different time point groups (0.5 h, 1 h, 3 h and 6 h after EMP exposure). To investigate the role of PKC inhibitor (H7) in EMP-induced BBB opening, 30 rats were divided randomly into 5 groups (n = 6) as follows. One sham group and four exposure groups (0.5 h after EMP exposure; 0.5 h after EMP exposure with H7 pretreatment, 3 h after EMP exposure, 3 h after EMP exposure with H7 pretreatment). To investigate the effect of H7 on the distribution of ZO-1, 18 rats were divided randomly into 3 groups (n = 6) as follows: one sham group, 3 h after EMP exposure group, 3 h after EMP exposure with H7 pretreatment group.

2.5. In vivo administration of PKC C inhibitor H7

H7, the inhibitor of PKC, was obtained from Sigma and dissolved in 0.3% NaCl (Maldonado et al., 1995). The animals in treatment groups were administered with 1 mg/kg of H7 (Li et al., 2000) by single i.p. injection 30 min before EMP exposure. (Li et al., 2000; Jio et al., 1989), sham group animals were administered with same volume of 0.9% NaCl.

2.6. Immunohistochemistry

The animals were anesthetized with 60 mg/kg i.p. of sodium pentobarbital, and then perfused transcardially with 100 ml of saline followed by 250 ml 4% formaldehyde in 0.01 M phosphate buffer at pH 7.4. After perfusion, brains were removed and post-fixed 24 h in the same solution, and then embedded in paraffin. 3–μm coronal sections were consecutively cut at the level of rostrum of corpus callosum, and one from every five sections was chosen for immunohistochemistry. The histological detection of endogenous albumin (molecular weight, 69 kD) extravasation was performed with goat anti-albumin primary antibody (1:500) and peroxidase conjugated secondary antibody. All slides were examined by two pathologists in a “blinded” fashion. The permeability of BBB in cerebral cortex was evaluated with a Kruskal–Wallis H test analysis method according to the scores of each rat. The scores were 0 in the sections with no albumin leakage microvessels; the scores were 1 in the sections with one albumin immuno-positive microvessel; the scores were 2 in the sections with two albumin immuno-positive microvessels; the scores were 3 in the sections with more than three albumin immuno-positive microvessel. The sum of the scores of 5 slides was the scores of each rat.

2.7. Isolation of microvessels from rat cerebral cortex

Brain microvessels were isolated from rat cerebral gray matter for determining the protein levels of PKC, ZO-1 as well as the distribution of ZO-1. The animals were anesthetized and killed by decapitation. Then, the brains were quickly dissected and the meninges and choroid plexuses were removed. Cerebral cortex was weighed and then homogenized in a five-fold volume of ice-cold buffer containing 147 mM NaCl, 4 mM KCl, 3 mM CaCl2, 1.2 mM MgCl2, 5 mM glucose and 15 mM Heps (pH 7.4).
2.8. Subcellular fractionation

The objective of this step was to resolve rat brain cortex microvessels endothelial cells into soluble (SOL) and cytoskeleton (CSK) fractions, the latter representing the subcellular compartment within which ZO-1 species was functionally associated with high-resistance TJ. Rat brain microvessels were incubated in 200 µL of Nonidet P-40 (NP-40) extraction buffer (25 mM N-[2-hydroxyethyl] piperazine-N’-[2-ethanesulfonic acid] (HEPES), pH 7.4, 150 mM NaCl, 4 mM EDTA, 1% (v/v) NP-40, 25 mM NaF, 1 mM NaN3, and 10 mM sodium pyrophosphate) containing 2% (v/v) protease inhibitor cocktail at 4 °C for 30 min with gentle rocking. Following centrifugation at 12,000 × g for 5 min, the supernatant (containing soluble proteins) was obtained. The insoluble CSK fraction was lysed into 100 µL of SDS extraction buffer (25 mM HEPES, pH 7.4, 1 mM EDTA, 1% SDS, 25 mM NaF, 10 mM sodium pyrophosphate) containing 2% (v/v) protease inhibitor cocktail at 4 °C for 30 min with gentle rocking. Following centrifugation at 12,000 × g for 5 min, the supernatant (containing solubilized CSK proteins) was obtained and diluted with an equal volume of NP-40 extraction buffer to reduce SDS concentration and equalize the volumes containing soluble and CSK fractions. Cellular equivalents of each fraction were analyzed by Western blot as described above (Song and Pachter, 2004).

2.9. Western blot

At different time points after EMP exposure, the rats were anesthetized as described above. The microvessels isolated from rat cerebral cortex were homogenized in a five-fold volume of ice-cold lysis buffer containing 50 mM Tris–HCl, 150 mM NaCl, 0.1% SDS, 1% NP-40, 0.5% sodium deoxycholate, 1% EDTA-2Na and 2% (v/v) protease inhibitor cocktail. It was shock frozen for 30 min on ice with gentle rocking. After that, the sample was centrifuged at 12,000 rpm for 5 min at 4 °C, and then the supernatant was stored at −80 °C. Bicinchoninic acid (BCA) protein assay was used for protein quantification and protein denaturation at 98 °C for 5 min at 5× sample buffer.

2.9.1. PKC Western blot analysis

Proteins (20 µg) were resolved on 10% denaturing SDS-polyacrylamide gel at 120 V for 90 min, and then transferred to polyvinyliden fluoride (PVDF) membranes. The non-specific binding sites were blocked by incubation for 2 h at room temperature in a blocking buffer containing 5% nonfat dry milk, then incubated for overnight at 4 °C with PKC, PKC-α and PKC-βII primary antibodies at a dilution of 1:200. Mouse monoclonal antibody directed against GAPDH (1:800) was used as a loading control. After washing three times with TBST, the membranes were then incubated with secondary antibody for 1 h at room temperature, and then the membranes were developed using enhanced chemiluminescence-detecting reagents (Western Lightening (Millipore, USA)).

2.9.2. ZO-1 Western blot analysis

Proteins (20 µg) were resolved on 6% denaturing SDS-polyacrylamide gel at 120 V for 90 min, and then transferred to PVDF membranes. The non-specific binding sites were blocked by incubation for 2 h at room temperature in a blocking buffer containing 5% nonfat dry milk, then incubated for overnight at 4 °C with rabbit polyclonal anti-ZO-1 primary antibody (1:200). GAPDH (1:800) was used as a loading control. The rest protocol is as same as PKC Western blot analysis.

The ability of H7 to block EMP-induced BBB permeability change suggested the possibility that EMP exposure activated PKC signaling. Since a marked increase in albumin extravasation was observed at 3 h after EMP exposure, we measured the protein level of PKC and PKCs isoforms (PKC-α, PKC-βII) in brain microvessels after exposure to EMP (200 kV/m, 200 pulses). Significant increases in PKC and PKC-βII were found in brain microvessels at 1 h after exposing rats to EMP compared with control. However, the protein level of PKC-α did not change within 6 h after EMP exposure. These data suggested that the effect of EMP on BBB permeability required PKC signaling related to elevated PKC, in particular, PKC-βII expression (Fig. 2).

2.10. Immunofluorescence

Microvessels were fixed in 4% (w/v) Triton X-100 at room temperature for 30 min, and then incubated in blocking buffer (5% normal goat serum, 0.5% Tween 20, 0.1% Triton X-100 in PBS) at room temperature for 1 h. After that, microvessels were incubated with rabbit polyclonal anti-ZO-1 antibody for overnight at 4 °C at the dilution of 1:500. After 3 × 10 min washing with PBS, the sections were incubated with FITC-conjugated goat anti-rabbit IgG (1:400 dilution) (ZF-0411, ZSGB-BIO) in the dark for at least 2 h at room temperature. Finally, samples were viewed directly and photographed under a Nikon TE 2000 fluorescent microscope.

2.11. Statistical analysis

All experimental data are expressed as mean ± S.E.M. Western blot data was examined by ANOVA followed by hoc pairwise comparisons and Dunnett’s test. The histopathologic evaluation data of BBB leakage was analyzed using Kruskal–Wallis test; multiple comparison method was Nemenyi test. Values of p less than 0.05 were considered statistically significant.

3. Results

3.1. PKC antagonist (H7) blocks EMP-induced changes in BBB permeability

Table 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Scores (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>6</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>NaCl + EMP</td>
<td>6</td>
<td>3.83 ± 0.75</td>
</tr>
<tr>
<td>H7 + EMP 0.5 h</td>
<td>6</td>
<td>2.67 ± 0.82</td>
</tr>
<tr>
<td>NaCl + EMP 3 h</td>
<td>6</td>
<td>9.83 ± 1.17</td>
</tr>
<tr>
<td>H7 + EMP 3 h</td>
<td>6</td>
<td>4.67 ± 1.03</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>4.20 ± 3.38</td>
</tr>
</tbody>
</table>

* p < 0.001 vs sham.

| H7 + NaCl + EMP group. |

3.2. EMP elevates PKC and PKCs isoforms protein expression

Previously, we found a decrease in ZO-1 protein level and alteration of ZO-1 distribution at 3 h after EMP exposure (Qiu et al., 2009). To determine whether translocation of TJ proteins was involved in EMP-induced and PKC-dependent increase in BBB permeability, we determined the distribution of ZO-1 in the cytoskeleton (CSK) fraction and Nonidet P-40-soluble (SOL) fraction. It was observed the protein level of ZO-1 decreased in CSK and SOL fraction at 3 h after EMP exposure compared with sham, in addition, the ratio of ZO-1 protein level in CSK fraction to SOL fraction decreased significantly after EMP exposure, PKC antagonists H7 pretreatment partly reversed EMP-induced ZO-1 translocation (Fig. 3).

3.3. EMP induces ZO-1 translocation via a PKC-dependent pathway

Fig. 4 showed the immunolocalization of ZO-1 in isolated brain microvessel segments from cerebral cortex. In control animals, ZO-1 showed a predominant pattern of continuous staining along the margins of cell–cell contact, after EMP exposure, ZO-1 lost its distribution in continuity and well defined filaments. Pretreatment
with PKC inhibitor H-7 partly reversed EMP induced the alteration of ZO-1 distribution in brain microvessels, which is consistent with the results of Western blot in cell fractions.

4. Discussion

The central nervous system is protected by BBB which control the entry of compounds into the brain, thereby regulating brain homeostasis. Many factors have been shown to induce deterioration of the BBB, such as encephalitis, hypoxia, posthypoxic reoxygenation (Fleegal et al., 2005), tumors (Liebner et al., 2000a), stroke (McCullough and Hum, 2003) and so on. As one of the main structures of BBB, TJ-associated proteins play a crucial role in maintenance BBB integrity. Our previous studies demonstrated that EMP could lead to BBB opening with the alteration of ZO-1 expression and/or distribution. But the mechanism is unclear. ZO-1 is a submembranous TJ-associated protein that belongs to the membrane-associated guanylate kinase (MAGUK) family. Its characteristic PDZ, SH3 and GK domains are enable ZO-1 to link transmembrane proteins, structural elements and signal transduction molecules (Kniesel and Wolburg, 2000).

Signaling pathways involved in TJ regulation include PKC, G-protein, serine-, threonine- and tyrosine-kinase, extra and intracellular calcium levels, proteases and cytokines. Common to most of these pathways is the modulation of cytoskeletal elements and the connection of TJ transmembrane molecules to the cytoskeleton (Wolburg and Lippoldt, 2002). We previously reported that EMP exposure altered BBB permeability in rats, and blood-testis barrier in mice (Ding et al., 2009a; Wang et al., 2008). In this study, we focused on the role of PKC in EMP-induced BBB opening. The results provided evidence that the PKC signaling pathway mediated the increase in BBB permeability of rats exposed to EMP.

The EMP effect on BBB permeability has been attributed to the alteration of TJ protein ZO-1 (Qiu et al., 2009). To identify whether EMP induced a redistribution of TJ proteins between CSK fraction and soluble fraction, and whether H7 pretreatment could

![Fig. 2](image1.png)

**Fig. 2.** Relative amount of PKC (PKC-α, PKC-βI, PKC-βII), PKC-α, and PKC-βII in cerebral cortex microvessels. Rats were sham or exposed to EMP at 200 kV/m for 200 pulses. The protein level of PKC and PKCs isozyme were measured by Western blot analysis. The experiment was performed at least twice. *p < 0.05 compared with sham.

![Fig. 3](image2.png)

**Fig. 3.** Protein level of ZO-1 in cytoskeleton (CSK) fraction and Nonidet P-40-soluble (SOL) fraction after EMP exposure with or without H7 pretreatment. The distribution ratio of ZO-1 (CSK/SOL) was surveyed by Western blot. The experiment was performed for three times. *p < 0.05 compared with sham group, Δp < 0.05 compared with EMP treatment only groups.

![Fig. 4](image3.png)

**Fig. 4.** Immunolabeling for ZO-1 in isolated rat cerebral cortex microvessels at 3 h after EMP exposure (200 kV/m, 200 pulses) with or without H7 pretreatment. Calibration bar, 20 μm.
reverse this processes, the CSK fraction and soluble fraction was extracted from brain microvessels. The protein level of ZO-1 was determined by Western blot. We observed a dissociation of ZO-1 from the cytoskeletal fraction at 3 h after EMP exposure (Fig. 3). The extensive disorganization and redistribution of ZO-1 in brain microvessels after EMP exposure was partly blocked by pretreatment with H7 (Fig. 4). These data suggested that translocation of TJ proteins was involved in EMP-induced and PKC-dependent increase in BBB permeability.

It was reported that BBB dysfunction was ameliorated when rats were pretreated with the PKC inhibitor H7 which inhibited PKC activity by binding to ATP-binding domain of PKC (Hidaka et al., 1984). In this study, there was a significant increase in the expression of total PKC and PKC-βII but not PKC-α after exposure to EMP. It has been shown that up-regulation of gene expression and isozyme synthesis may contribute to the overall increase in PKC enzymatic activity (Guo et al., 2003; Fleegal et al., 2005). Therefore, these data suggested that EMP may increase PKC activity by enhancing the PKC protein expression. Moreover, the activation of PKC preceded the EMP-associated alterations of albumin extravasation. In addition, PKC-βII is one of the sensitive PKCs isofrom to EMP exposure. A substantial body of experimental data indicates that PKC regulates paracellular permeability in endothelial monolayers (Ferro et al., 2000).

Functionally, PKC has been frequently referred to as a key mediator of microvascular permeability under stimulated conditions. There are several possible mechanisms by which PKC may regulate BBB permeability. One possible mechanism is direct regulation (phosphorylation) of the TJ proteins by PKC. It has been shown that PKC could regulate the phosphorylation of occludin (Anna et al., 2001; Suzuki et al., 2009), and occludin phosphorylation may alter its interactions with intracellular scaffolding proteins (Krizbai and Deli, 2003) and its localization (Anna et al., 2001; Song and Pachter, 2004). PKC-α has been shown to be co-localized with ZO-1 in Madin–Darby canine kidney (MDCK) and human Cau-casian colon adenocarcinoma-2 (Caco02) cell lines (Dodane and Kachar, 1996). PKC-α and PKC-β signaling was shown to regulate toxin A-mediated paracellular permeability changes and ZO-1 translocation (Chen et al., 2002). ZO-1 is a target of phosphorylation by PKC-β (Avila-Flores et al., 2001). In this study, we found that the alterations in ZO-1 protein localization occurred in cerebral cortex microvessel after EMP exposure. ZO-1 is a scaffolding protein connecting with actin filaments with TJ fibril proteins, claudins, and occludin (Mitic et al., 2000). Therefore, the alteration in ZO-1 protein localization is suggested to be associated with EMP-induced BBB opening. Pretreatment with PKC inhibitor H7 improved the alteration of ZO-1 distribution in microvessels (Fig. 4) as well as partly prevented EMP-induced BBB opening. These data support the hypothesis that PKC may directly regulate the structure and function of TJ proteins.

One possible explanation is that PKC may be indirectly Altering the TJ proteins by activating a downstream intracellular signaling pathway that in turn directly affects TJ proteins. Previous studies have demonstrated that changes in NO and intracellular Ca²⁺ contribute to an increased paracellular permeability induced by H and H⁺/R. Increases in the expression of the Ca²⁺-dependent isozymes PKC-βII, which can regulate NOS, suggested that PKC might interact with this signaling molecule to contribute to BBB permeability alterations (Ban et al., 2003). Furthermore, PKC may participate in the modulation of gene expression by other pathways. For example, PMA can activate PKC to elicit p44/42 MAPK activation. Phosphorylated MAPK can activate gene regulation protein Elk-1 by phosphorylation. Activated Elk-1 is bond to a short DNA sequence (serum response element, SRE), and regulate gene expression with another factor (serum response factor, SRF) (Qiu and Leslie, 1994). Another pathway is that PKC can mediate NF-κB activation or phosphorylate Ik-B, and release gene regulation protein NF-κB which can active definite gene transcription after enter cellular nucleus (Ward et al., 1999). Besides PKC, recent studies demonstrated that increased activation of matrix metalloproteinase (MMPs), especially MMP-2 and MMP-9, could destruct the integrity of TJ by degradation TJ proteins occludin, ZO-1 and claudin (Yang et al., 2007; Liu et al., 2009). And monocyte chemoattractant protein-1 (MCP-1) leads to opening the BBB through changing the distribution and diminishing the expression of ZO-1 and ZO-2 (Sheth et al., 2003).

Our study showed that PKC could affect the distribution of ZO-1. H-7 is one of the most potent inhibitors in inhibiting PKC (Hidaka et al., 1984), and it has been used for inhibiting PKC to improve the permeability of BBB widely (Joo et al., 1989). After using H7 before EMP exposure, the permeability of BBB was improved compared with EMP exposure only, in addition, the ratio of CSK/SOL was elevated at 3 h after EMP exposure, meanwhile, the immunofluorescence staining results showed that the alteration of residual ZO-1 location in brain microvessels was also lessened compared with EMP exposure only.

In conclusion, this study demonstrates that the EMP-induced increase in PKC expression in rat brain cortex plays an important role in altering BBB permeability, and PKC signaling is involved in EMP-induced ZO-1 translocation in rat BBB.

Conflict of interest statement

There are none.

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