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

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ABSTRACT

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-βII (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.

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). Expression of these proteins is frequently modified in many conditions such as inflammation, ischemia-reperfusion injury, sepsis, thermal injury, diabetes, and atherosclerosis (Weiss et al., 2009).

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 D-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

Abbreviations: BBB, blood–brain barrier; ZO, zonula occludens proteins; EMF, electromagnetic field; BMEC, brain microvascular endothelium cell; MCP-1, monocyte chemoattractant protein-1; TJ, tight junction; PKC, protein kinase C; PKA, cyclic AMP (cAMP)-dependent protein kinase; PKG, cyclic GMP (cGMP)-dependent protein kinase; MAPK, mitogen-activated protein kinase; TER, transendothelial electrical resistance; GAPDH, glyceraldehyde phosphate dehydrogenase; SOS, semiconductor opening switch; NP-40, Nonidet P-40; HEPES, N-[2-hydroxyethy] piperazine-N′-[2-ethansulfonic acid]; CSK, cytoskeleton; SOL, Nonidet P-40-soluble; PVDF, polyvinylidene difluoride; BCA, bicinchoninic acid; MAGUK, membrane-associated guanylate kinase; MDCK, Madin–Darby canine kidney; MMP, matrix metalloproteinase; EMP, electromagnetic pulse; H7, 1(5-chloronapthalene-1-sulfonyl)-1H-hexahydro-14-diazepine; SRE, serum response element; SRF, serum response factor.

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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: Ca^{2+}/phospholipid-dependent conventional PKC-α, -β, and -γ; Ca^{2+}-independent novel PKC-δ, -ε, -η, and -θ; and phospholipid-independent atypical PKC-ζ and -λ (Tanaka and Nishizuka, 1994). It was shown that PKC-α, -β, -γ, -δ, -ε, -η, and -ζ isoforms 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 (61-7300) was obtained from Zymed Biotechnology (USA). Mouse monoclonal antibody directed to PKC (BM0401), rabbit polyclonal antibody directed to PKC-α (BA1355), and rabbit polyclonal antibody directed to PKC-βII (BA1358) were purchased from Boster Biological Technology, Ltd (Wuhan, China). As a loading control for Western blot, mouse monoclonal antibody directed to GAPDH was purchased from ZSGB-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 ZSGB-BIO (Beijing, China). Molecular weight standards for Western blot were obtained from Fermentas (SM1841, USA). PKC inhibitor H7 (1(5-chloronapthalene-1-sulfonyl)-1H-hexahydro-1,4-diazepine) (I6891) 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 wave form 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.

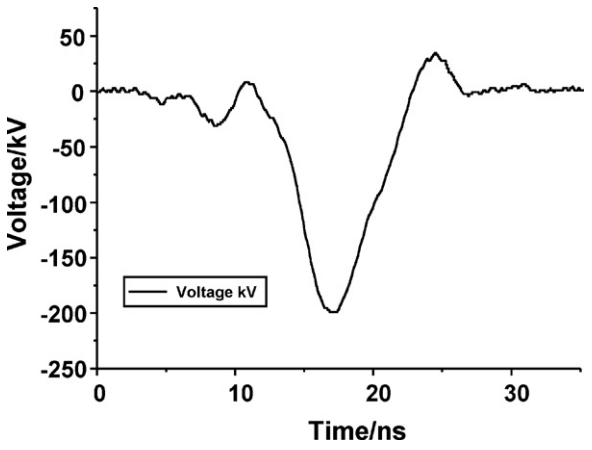

Fig. 1. Output wave form from EMP generator.

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 4 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 0.9% NaCl (Maldonado et al., 1995). The animals in treatment groups were administrated with 1 mg/kg of H7 (Li et al., 2000) by single i.p. injection 30 min before EMP exposure (Li et al., 2000; Joó et al., 1989), sham group animals were administrated with same volume of 0.9% NaCl.

2.6. Immunohistochemistry

The animals were anaesthetized 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 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 microvessel; 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 $CaCl_2$, 1.2 mM $MgCl_2$, 5 mM glucose and 15 mM Hepes (pH 7.4).