Original Article

The Effects of Electromagnetic Pulse on the Protein Levels of Tight Junction Associated-Proteins in the Cerebral Cortex, Hippocampus, Heart, Lung, and Testis of Rats*

QIU LianBo[1], CHEN Chen[1,†,#], DING GuiRong[2,‡], ZHOU Yan[2], and ZHANG MengYao[3]

1.Department of Blood Transfusion, Xijing Hospital, Fourth Military Medical University, Xi'an 710032, Shannxi, China; 2.Department of Radiation Medicine, Faculty of Preventive Medicine, Fourth Military Medical University, Xi'an 710032, Shannxi, China; 3.Department of Chemistry, School of Pharmacy, Fourth Military Medical University, Xi'an 710032, Shannxi, China

Abstract

Objective To investigate changes in the expression of tight junction (TJ) proteins in the cerebral cortex, hippocampus, heart, lung, and testes of rats after exposure to electromagnetic pulse (EMP).

Methods Eighteen adult male Sprague-Dawley rats were divided into sham and exposure groups. The exposure groups received EMP at 200 kV/m for 200 pulses with a repetition rate of 1 Hz. The expression of TJ proteins (ZO-1, occludin, actin) in the several organs was examined by western blotting.

Results ZO-1 levels in the cerebral cortex decreased 1 h and 3 h after EMP exposure compared with sham group (P<0.05). No significant difference was observed for occludin and actin. ZO-1 levels in the hippocampus increased 1 h and 3 h post-exposure (P<0.05), and occludin decreased after 3 h (P<0.05); however, actin was unaffected. ZO-1 levels in the heart increased 3 h post-exposure (P<0.05), occludin decreased 3 h post-exposure (P<0.05), and actin increased 1 h and 3 h post-exposure (P<0.05). ZO-1, occludin and actin levels in the lung decreased compared with those in the sham group (P<0.05). ZO-1 and occludin levels in the testes decreased 1 h and 3 h post-exposure (P<0.05), but actin showed no significant change.

Conclusion Exposure to EMP altered the expression levels of TJ proteins, particularly ZO-1, in the organs of adult male rats, which may induce changes in barrier structure and function.

Key words: Electromagnetic pulse; Tight junction; ZO-1; Occludin; Actin

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INTRODUCTION

With the development of modern scientific technology, electromagnetic pollution is becoming a critical environmental and physical factor that impacts on human health. Electromagnetic waves have two forms, a continuous wave and a pulse wave, also known as an electromagnetic pulse (EMP). The EMP used in this study was a short high-voltage pulse with an extremely fast rising time and a broad bandwidth. This type of signal can be generated by a nuclear bomb explosion[1]. EMP signals also exist in certain work places, for example the Pulse Power Technology Lab, where equipment such as high pressure gas switches and Tesla transformer

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#Correspondence should be addressed to CHEN Chen. Tel: 86-29-84775465, Fax: 86-29-84775465, E-mail: YYchenchen@163.com

†Contribute equally to the first author. ‡Co-corresponding author.

Biographical note of the first author: QIU LianBo, male, born in 1982, Doctor.

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generators produce strong electrical fields. The unusual properties of EMP have raised concerns about their biological effects and possible health hazards, especially with respect to military personnel and workers or researchers that may be exposed to this kind of electromagnetic field. However, the biological effects of EMP remain unclear[2].

It is thought that EMP affects the cells of an organism to a greater extent than continuous waves, damaging the macromolecules that comprise the cell membrane and affecting cell function[1]. Tight junctions (TJ) are found in many organs and organisms, for example in the skin, brain, lung, heart, liver, testis, and amphiblestrodes. TJ are located at the gap between adjacent cells, joining their cytoskeletons, and are essential for the barrier function of the epidermis and endothelial cells[3]. Endothelial TJ differ from epithelial TJ in terms of their morphological and molecular properties and the fact that endothelial TJ are more sensitive to the microenvironment than epithelial TJ[4]. However, these two types of TJ have similar molecular structures. TJ comprise complexes of transmembrane proteins and sub-membranous components that link the transmembrane components to the actin cytoskeleton[5]. Genetic and biochemical studies in invertebrates and vertebrates indicate that TJ proteins play an important role in the establishment and maintenance of apico-basal polarity[6]. Several TJ-associated protein components have been identified, including occludin[7], Claudin-1, Claudin-5[8], the sub-membranous components, ZO-1[9], ZO-2[10], and ZO-3[11], and cytoskeleton microfilaments (actin). The transmembrane protein occludin is an excellent candidate sealing protein and is bound to ZO-1 and ZO-2 on the cytoplasmic membrane surface. The functions of ZO-1 and ZO-2 are suggested from those of their invertebrate homologues. Occludin was the first TJ transmembrane protein found to directly participate in the formation of TJ in brain microvascular endothelial cells[12]. The carboxyl-terminal 150 amino acids of occludin can link directly with actin and ZO-1/ZO-2/ZO-3[13]. ZO-1 binds to cytoskeletal actin and actin binding proteins through its carboxyl terminus[13]. Thus, occludin and ZO-1 are the most important proteins within the TJ structure. Dynamic regulation of peri junctional actin has emerged as a unifying hypothesis for controlling paracellular permeability[14].

The expression of TJ proteins is often modified in many conditions such as inflammation, ischemia-reperfusion injury, sepsis, thermal injury, diabetes, and atherosclerosis[15]. Previously, we found that EMP increases the permeability of the blood-brain barrier (BBB) within the frontal lobe of the rat brain. Changes in ZO-1 expression (decreasing protein levels and changes in localization) and the PKC signaling pathway might play an important role in this process[16-17]. However, it is unclear whether EMP causes changes in TJ proteins within other organs. Therefore, in the present study (which was limited by our laboratory conditions) we investigated changes in the expression levels of several TJ-associated-proteins (ZO-1, occludin and actin) in the cerebral cortex, hippocampus, heart, lung, and testes of rats after exposure to EMP.

MATERIALS AND METHODS

Reagents

SDS, acrylamide, and bisacrylamide were purchased from Sigma-Aldrich Corporation (USA). Rabbit polyclonal antibodies against ZO-1 (61-7300) and occludin (71-1500) were obtained from Zymed Biotechnology (USA). The rabbit polyclonal antibody against actin (sc-1616-R) was obtained from Santa Cruz (USA). The mouse monoclonal antibody against GAPDH was purchased from ZSGB-BIO (Beijing, China) and used as a loading control for the western blots. Molecular weight standards for western blotting were obtained from Fermentas (SM1841, USA).

EMP Exposure Apparatus

The all-solid-state nanosecond generator developed and tested at the Northwest Institute of Nuclear Technology in Xi'an, China has been described elsewhere[18]. Briefly, the generator comprises three relatively independent units: a resonant charging unit, a magnetic pulse compression unit, and a semiconductor opening switch (SOS) unit. The resonant charging unit regulates the primary pulse from the mains power, and the magnetic pulse compression unit (including the magnetic saturation pulse transformers and the magnetic switches) increases the pulse voltage and compresses the pulse width. Because of the effect of the forward and reverse pumping current, the SOS cuts off the current immediately. The energy stored in the inductor is eventually transferred to the load;

meanwhile, a high voltage and short pulse output is achieved. The electric field within an exposure area of 30 cm × 30 cm × 30 cm is uniform. EMP pulses (200 kV/m) with a 3.5 ns rising time, a 14 ns pulse width, and a 1 Hz repetitive rate were used in this experiment.

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, China). Male Sprague-Dawley rats weighing 200-250 g were obtained from the 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 free access to semi-purified rat chow and pre-prepared drinking water. The animals were either sham or whole-body exposed to 200 pulses of 1 Hz EMP at 200 kV/m. During exposure, the rats were awake and unrestrained in the exposure chamber. Body temperature was measured immediately before and after EMP exposure, which caused a rise in rectal temperature of <0.2 °C.

Experimental Groups

Eighteen rats were divided randomly into the sham group and the exposure groups. The exposure groups were further divided into two different time points (1 h and 3 h after EMP exposure).

Western Blotting

At different time points after EMP exposure, rats were anesthetized with 60 mg/kg i.p. of sodium pentobarbital. The cerebral cortex, hippocampus, heart, lung and testes were homogenized in a 5-fold volume of ice-cold lysis buffer containing 50 mmol/L Tris-HCl, 150 mmol/L NaCl, 0.1% SDS, 1% NP-40, 0.5% sodium deoxycholate, 1% EDTA-2Na and 2% (v/v) protease inhibitor cocktail. Samples were schizolyzed for 30 min on ice with gentle rocking followed by centrifugation at 12 000 rpm for 5 min at 4 °C. The supernatant was then stored at -80 °C. A bicinchoninic acid (BCA) protein assay was used for protein quantification after the protein was denatured at 98 °C for 5 min at 5× sample buffer.

Proteins (30 μg) were resolved on 6% (for ZO-1), 10% (for occludin) or 12% (for actin) denaturing SDS-polyacrylamide gels at 120 V for 90 min, and then transferred to polyvinylidene difluoride (PVDF) membranes. Non-specific binding sites were blocked by incubation for 2 h at room temperature in blocking buffer containing 5% non-fat dry milk, and then incubated overnight at 4 °C with antibodies against ZO-1, occludin or actin at a dilution of 1:200, 1:200, and 1:800, respectively. A mouse monoclonal antibody against GAPDH (1:800) was used as a loading control. After washing three times with TBST, the membranes were incubated with the secondary antibody for 1 h at room temperature, and the membranes developed using enhanced chemiluminescence detection reagents (Western Lightening; Millipore, USA).

The intensity of each target band was semi-quantified using Quantity One imaging software (Bio-Rad Laboratories, Hercules, CA, USA). Data were analyzed using the ratio of the densitometric intensity of the target bands and GAPDH bands on the same membrane.

Statistical Method

All experimental data were expressed as the mean±S.E.M. Western Blotting data were examined using one-way ANOVA followed by hoc pairwise comparisons and Dunnett's test. $P<0.05$ was considered to be statistically significant.

RESULTS

ZO-1 in the Cerebral Cortex Decrease after EMP Exposure

ZO-1 levels in the cerebral cortex decreased significantly 3 h after EMP exposure compared with those in the sham group ($P<0.05$). However, the levels of occludin and actin showed no significant change ($P>0.05$, Figure 1).

ZO-1 Levels Increase and Occludin Levels Decrease in the Hippocampus after EMP Exposure

The level of ZO-1 in the hippocampus increased significantly 1 h and 3 h after exposure to EMP compared with that in the sham group ($P<0.05$); however, the level of occludin decreased significantly 3 h after EMP exposure ($P<0.05$). Actin levels were unchanged (Figure 2).

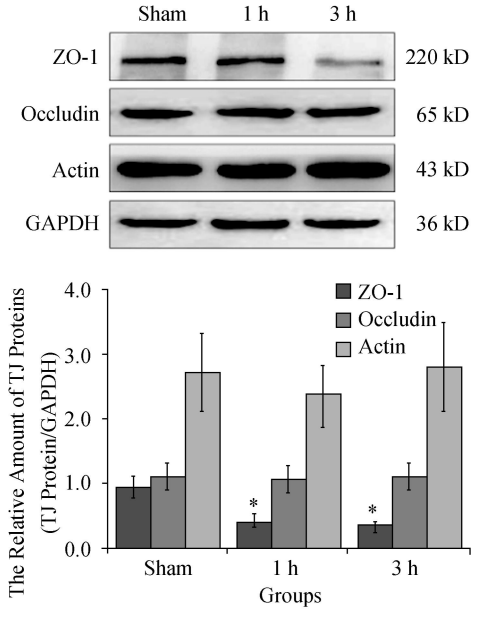

Figure 1. Relative amounts of ZO-1, occludin and actin in the cerebral cortex of rats in the sham group and 3 h after EMP exposure (200 kV/m, 200 pulses). $^{*}P<0.05$ compared with sham (ZO-1).

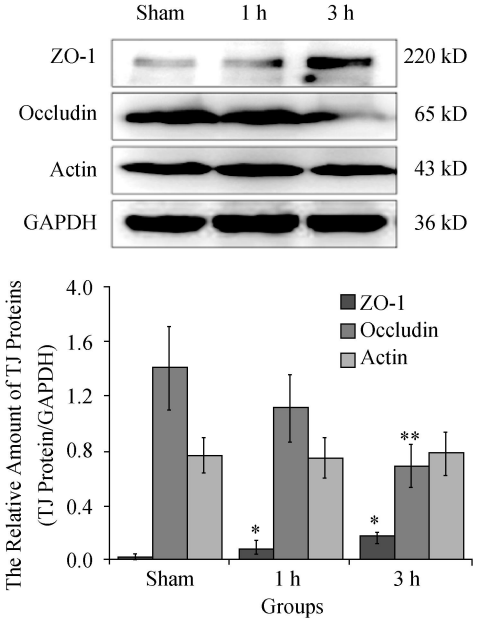

Figure 2. Relative amounts of ZO-1, occludin and actin in the hippocampus of rats in the sham group and 1 h and 3 h after EMP exposure (200 kV/m, 200 pulses). $^{*}P<0.05$ compared with sham (ZO-1). $^{**}P<0.05$ compared with sham (occludin).

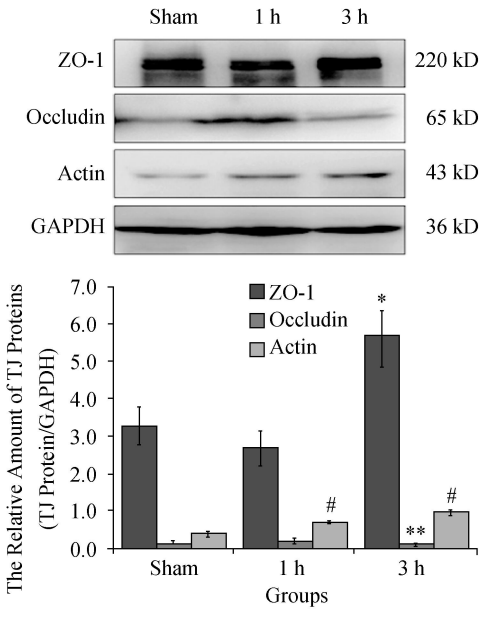

Figure 3. Relative amounts of ZO-1, occludin and actin in the heart of rats in the sham group and 1 h and 3 h after EMP exposure (200 kV/m, 200 pulses). $^{*}P<0.05$ compared with sham (ZO-1). $^{**}P<0.05$ compared with sham (occludin). $^{\#}P<0.05$ compared with sham (actin).

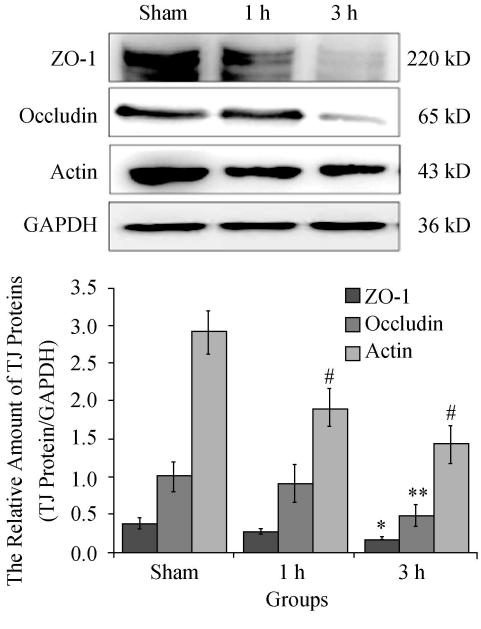

Figure 4. Relative amounts of ZO-1, occludin and actin in the lung of rats in the sham group and 1 h and 3 h after EMP exposure (200 kV/m, 200 pulses). $^{*}P<0.05$ compared with sham (ZO-1). $^{**}P<0.05$ compared with sham (occludin). $^{\#}P<0.05$ compared with sham (actin).

ZO-1 and Actin Levels Increase and Occludin Levels Decrease in the Heart after EMP Exposure

The level of ZO-1 in the heart increased 3 h post-exposure, and the level of actin increased 1 h and 3 h after EMP exposure compared with those in the sham group (P<0.05); however, occludin levels decreased significantly 3 h after EMP exposure (P<0.05, Figure 3).

The Levels of ZO-1, Occludin and Actin Decrease in the Lung after EMP Exposure

The levels of ZO-1, occludin and actin in the lung all decreased 3 h after EMP exposure compared with those in the sham group (P<0.05, Figure 4).

The Levels of ZO-1 and Occludin Decrease in the Testes after EMP Exposure

The levels of ZO-1 and occludin in the rat testes were decreased 1 h and 3 h after EMP exposure compared with those in the sham group (P<0.05); however, the level of actin did not change significantly (P>0.05, Figure 5).

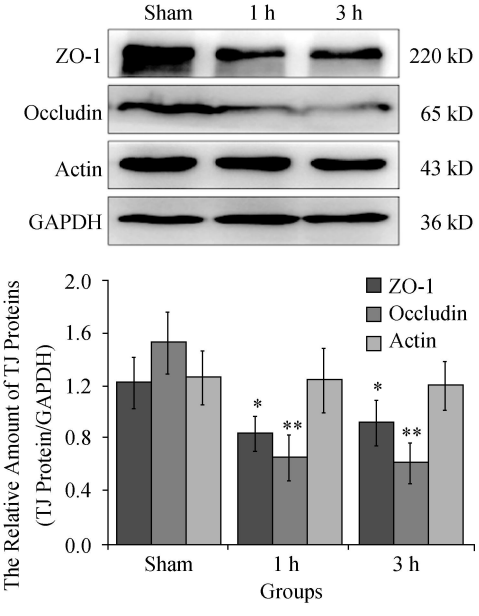

Figure 5. Relative amounts of ZO-1, occludin and actin in the testes of rats in the sham group and 1 h and 3 h after EMP exposure (200 kV/m, 200 pulses). $^{*}P$<0.05 compared with sham (ZO-1). $^{**}P$<0.05 compared with sham (occludin).

DISCUSSION

In recent years, the potential health risks from exposure to electromagnetic fields (EMF) have become a major concern. Therefore, it is important to study the biological effects of EMF that lead to health impairment. To date, research has focused on the biological effects of continuous electromagnetic waves whereas few studies have examined EMP. EMP exposure may cause changes in the biological functions of several organs. Barrier structures play an important role in biological function, but little is known about the effects of EMP on the levels of the TJ proteins that make up these barrier structures. Previously, we found that EMP exposure caused ZO-1 levels in rats to decrease, but occludin and actin levels were unaffected[16-17]. Wang et al.[19] also observed similar results in mice.

The formation of stable cell-cell contacts is required to generate barrier-forming sheets of epithelial and endothelial cells. During various physiological processes, such as tissue development, wound healing and tumorigenesis, cellular junctions are thought to allow the release, or the incorporation, of individual cells. Cell-cell contact is regulated by multiprotein complexes localized at specific structures along the lateral portion of the cell junctions, including TJs and adherens junctions (AJs), which are targeted to these sites through their association with cell adhesion molecules[20]. A major function of AJs is to connect cells and regulate tissue formation and morphogenesis during development and to maintain solid tissues in adult organisms[21]. TJs have two major functions. First, they regulate the paracellular permeability of the epithelial sheet to ions and small solutes, which is an organ-specific function that varies in different epithelia, depending on the specific requirements of the organ[22-23]. Second, they help to form a physical barrier that prevents intra-membrane diffusion of lipids and proteins; a rather cell-autonomous function that is necessary if a cell is to maintain an asymmetric distribution of membrane components and develop membrane polarity[24].

In previous studies of the cerebral cortex[16-18, 25], we found that the permeability of the BBB permeability and the level of TJ proteins began to change 0.5-1 h after EMP exposure, with the greatest changes seen after 3 h. Permeability and TJ formation then gradually recovered. To study whether the effect of EMP on TJ proteins in other organs is similar to those observed in the cerebral cortex, we chose two time points (1 h and 3 h after EMP exposure) at which to investigate the protein levels of three typical TJ proteins—ZO-1, occludin

and actin. Any alteration in TJ protein expression may induce changes in the barrier structure and function.

As mentioned, EMP causes opening of the BBB within the cerebral cortex and hippocampus (and other areas of the brain), but the time scales involved are different[26]. This phenomenon is observed in many conditions. For example when the rat brain is damaged, the permeability of the hippocampus is significantly less than that of the cerebral cortex[27]. However, the underlying mechanisms remain unclear. In this study, we found that ZO-1 protein levels decreased in the rat cerebral cortex, but increased in the hippocampus 1 h and 3 h after EMP exposure. The level of occludin did not change in the cerebral cortex, but decreased significantly in the hippocampus 3 h after EMP exposure. Previously, we found that the mechanism of EMP-induced BBB opening within the cerebral cortex maybe related to decreasing levels and altered localization of ZO-1, and that the PKC and/or other signaling pathways might modulate this process[17-18, 25]. We speculated that the difference in TJ protein expression (ZO-1 and occludin) may be one of the mechanisms involved.

We obtained similar results to those in the hippocampus using heart tissue, in which the expression of ZO-1 and occludin increased. The heart is very susceptible to EMF. According to epidemiological surveys, the cardiac function of people exposed to electromagnetic waves may be affected to different degrees[28]. Electromagnetic radiation can damage the heart, (e.g. causing left ventricular transmural degenerative changes) and the number of lesions increases with time[29]. The myocardial structure is severely damaged by high power microwaves (HPM) in a dose-dependent manner. Bcl-2/bax, p53, and C-fos are all involved in myocardial cell apoptosis induced by HPM[30]. After high power pulse microwave (HPPM) and EMP irradiation, cardiomyocytes pulse at a slower rate or stop, cell conformation is abnormal, cell viability declines and the percentage of apoptotic and necrotic cells increases significantly. The cell membranes also contain pores of unequal size, and lose their penetration characteristics[30]. Indeed, electroporation is one of the most critical mechanisms that explain the athermal effects of electromagnetic radiation[31]. Pulse microwaves may have a marked influence on membrane protein structures. The secondary structures of membrane proteins were also altered by irradiation. The percentage of alpha-helix and beta-pleated sheet structures decreases, and the secondary membrane proteins become increasingly disordered. All these changes correlate with the irradiation dose[32]. Changes of TJ protein expression may be associated with the above-mentioned alternations in the membrane structure and heart function.

The testes are highly sensitive to electromagnetic radiation, which may cause structural and functional damage, including reductions in sperm motility, increasing levels of abnormality and ultra-structural alternations. Leydig cells are very susceptible to EMP irradiation. EMP irradiation causes significant damage to the structure and function of Leydig cells in mice, which is likely to affect sexual function and sperm production[33]. EMP exposure may also increase the permeability of the blood-testis barrier (BTB) in mice[19]. Compared with the sham group, the expression of ZO-1 and TGF-beta3 significantly decreased in EMP-exposed mice. This was accompanied by unevenly stained vimentin microfilaments and increased serum as antibody levels[34]. EMF also changes the activity of the sex glands in rats[35]. In our study, we found that the expression of ZO-1 and occludin decreased 1 h and 3 h after EMP exposure, and that this was associated with alternations in BTB permeability and testis function.

Epithelial and endothelial TJs are both critical elements of the permeability barrier and are required to maintain discrete compartments within the lung. There are few studies of the biological effects of electromagnetic irradiation on the animal and/or human lung. Zhang et al.[36] found that exposure to 1 800 MHz radiofrequency electromagnetic fields (RF EMF) (SAR, 3.0 W/kg) for 24 h induced DNA damage in Chinese hamster lung (CHL) cells. Extremely low frequency electromagnetic fields (ELF EMF) can also suppress gap-junctional intercellular communication (GJIC), which may result from an EL EMF-induced shift of connexin 43 (Cx43) from the intercellular junctions to the cytoplasm[37]. In the present study, the expression ZO-1, occludin and actin in the lung all decreased after exposed to EMP.

In conclusion, our data suggest that EMP exposure (200kV/m, 200 pulses) changes the expression of TJ proteins in the cerebral cortex, hippocampus, heart, lung and testes of rats. This alteration in TJ protein levels may be one of the mechanisms underlying EMP-induced functional

changes observed in several organs. Because our research is still at a preliminary stage, many questions remain to be answered: for example, whether TJ proteins show different changes in different organs. These studies are ongoing.

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