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Original Research

Protein kinase C-ε contributes to a chronic inhibitory effect of IL-1β on voltage-gated sodium channels in mice with febrile seizure

Jinli Wang[1], Fenfen Xu[1], Yuan Zheng[1], Xu Cheng[2], Piaopiao Zhang[2] and Hongyang Zhao[1,*]

[1] *Department of Pediatrics, Jinan Central Hospital Affiliated to Shandong University, Jinan City, Shandong Province, 250013, P. R. China*

[2] *Graduate School of Taishan Medical University, Taian City, Shandong Province, 271016, P. R. China*

*Correspondence: zhy1587@zxyy.cn (Hongyang Zhao)

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This study aimed to understand the role of Interleukin-1β in mouse febrile seizures. To investigate the chronic effects of raised Interleukin-1β on seizures, the sodium currents of hippocampal neurons were recorded by whole-cell voltage clamp. Interleukin-1β inhibited sodium currents in mouse hippocampal neurons and verified that protein kinase C epsilon contributed to the effect of Interleukin-1β exposure. The inhibitory effect was also identified in neurons from a protein kinase C epsilon null mutant mouse. Action potentials were recorded using a ramp depolarizing current. Peak spike depolarization was significantly reduced by Interleukin-1β treatment, and was abolished following the administration of a protein kinase C epsilon inhibitor, εV1-2. However, neither Interleukin-1β nor εV1-2 had any significant effect on spike threshold. Interleukin-1β reduced the amplitude of action potentials due to its inhibitory effect on sodium channels. This is hypothesised to decrease the release of presynaptic transmitters of neuroexcitability, thus exerting a neuroprotective role in excitotoxicity. To ascertain the role of protein kinase C epsilon on febrile seizures *in vivo*, a heated water-bath model was used to identify susceptible mice. It was found that protein kinase C epsilon reduced susceptibility to, and frequency of, febrile seizure onset. This may be related to the neuroprotective effect of Interleukin-1β on hippocampal neurons.

Keywords

Febrile seizure; IL-1β; hippocampus; voltage-gated sodium channel; PKC-ε

1. Introduction

Animals that exhibit febrile seizure (FS) reportedly have high levels of hippocampal Interleukin-1β (IL-1β), and intracerebroventricular (ICV) injection of IL-1β increases the number of animals with FS (Heida et al., 2009). Clinical studies suggest that inflammation, which is intrinsic to the fever response, is involved in the generation of FS (Choy et al., 2014). Inflammatory cytokines play functional roles in neuronal excitability and, specifically, receptors for IL-1β have been found in the hippocampus at a high concentration (Dubé et al., 2007). The expression of IL-1β increases in the central nervous system, which is concerned with FS mechanisms (Haspolat et al., 2002). The expression of IL-1β and its mRNA increases in the cortex, hypothalamus and hippocampus following various artificially induced seizures (Dhote et al., 2007; Donnelly et al., 1999; Eriksson et al., 2000). In a previous study, mice that lacked the IL-1β type 1 receptor were significantly more resistant to FS, which suggests a role for IL-1β in seizure generation (Dubé et al., 2005). Prolonged experimentally generated FS induces a long term increase in hippocampal excitability, which may enhance susceptibility to future seizures (Brewster et al., 2002). Cultured hippocampal cells exposed to IL-1β had increased influx of Ca^{2+} that could lead to potentiated excitability and putatively allow IL-1β to contribute to FS (Dubé et al., 2005; Heida and Pittman, 2005; Viviani et al., 2003). Therefore, hippocampal neurons were used in *in vitro* experiments to explore the underlying mechanism of IL-1β in the progress of FS.

As previously reported, mechanisms for FS involve alterations in molecular and functional properties, such as neurotransmitter receptors (Sanchez et al., 2001; Zhang et al., 2004) and voltage-gated ion channels (Brewster et al., 2002; Chen et al., 2001). Among them, IL-1β is capable of suppressing sodium channel β-subunit expression in epithelial cells (Choi et al., 2007). A brief exposure of IL-1β led to reduction of Na^+ current, whereas chronic IL-1β treatment potentiated Na^+ currents in trigeminal nociceptive neurons (Liu et al., 2006). IL-1β treatment also inhibits voltage-gated Na^+ channels by reducing currents in cortical neurons via its receptor in a time- and dose-dependent manner, but does not affect voltage-dependent activation and inactivation (Zhou et al., 2011). IL-1β inhibits the release of excitatory transmitters, such as glutamate, that suppress the excitotoxicity of neurons (Murray et al., 1997). However, in hippocampus, the interactions of IL-1β and Na^+ channels are unclear.

The protein kinase C (PKC) family is highly expressed in neuronal tissues. IL-1β can sensitize sensory neurons to heat via IL-1R1 signaling through PTK-mediated downstream activation of PKC and subsequent targeting of heat-transducing vanilloid re-

ceptors such as VR-1 or VRL-1 (Obreja et al., 2002). In alveolar epithelial cells, IL-1β induced secretion of β-calcitonin gene-related peptide depends on PKC, p38 MAPK and NF-κB (Li et al., 2004). Moreover, IL-1β inhibited Na^+ channels in alveolar epithelial cells mediated by p38 MAPK and suppressed Na^+ currents in cortical neurons dependent on PKC and p38 MAPK (Roux et al., 2005; Zhou et al., 2011). Thus, further exploration of the interplay between IL-1β and PKC in hippocampal neurons is a key step to further elucidation of these interactions.

PKC includes conventional (α, βI / βII, and γ), novel (δ, ε, η, θ, and μ), and atypical (ζ, λ, and ι) isoforms, which show subtle differences in enzymology, cellular/subcellular localization and tissue expression (C Tanaka and Nishizuka, 1994). The PKC-ε isoform exerts significant functions in different mechanisms and pathological states or systems. Firstly, in nervous system, PKC-ε is principally responsible for sensitization by bradykinin of the heat response in nociceptors (Cesare et al., 1999) and PKC-ε is involved in neuronal death induced by oxidative stress (Jung et al., 2004). Secondly, it has been reported that PKC-α and -ε down-regulate cell surface sodium channels in adrenal chromaffin cells (Yanagita et al., 2000). Moreover, activation of the neurokinin-1 receptor potentiates the $Na_V1.8$ sodium current via a PKC-ε-dependent signaling pathway, which may participate in the generation of inflammatory hyperalgesia (Cang et al., 2009). Further, functional regulation of the $Na_V1.8$ mRNA by PKA and PKC-ε in primary sensory neurons is important for the development of the peripheral pro-nociceptive state (Villarreal et al., 2009). PKC-ε also mediates the regulation of Na^+ channels by PKC isoform-specific mechanisms and plays important roles in both Na^+ channels and neurons, but related studies are seldom on hippocampus cells.

To investigate the chronic effects of IL-1β on Na^+ channels in hippocampal neurons, Na^+ currents were recorded in PKC-ε null mutant mice by whole-cell voltage clamp. Action potentials were recorded using a ramp depolarizing current. To ascertain the role of PKC-ε in FS *in vivo*, a heated water-bath model of induced FS in mice was prepared and the effect of PKC-ε on the susceptibility and frequency of FS onset evaluated.

2. Methods

2.1 Hippocampal neuron preparation

Hippocampal neuron preparation procedures followed Kay and Wong (1987a) and Surmeier et al. (1992a), for the culturing of guinea pig pyramidal and neo-striatonigral neurons, respectively. C57BL/6J mice (6-12 weeks old) were anesthetized using halothane and decapitated. The brain was quickly removed and transversely sliced (500 μm). The slices were incubated in $NaHCO_3$ buffered Earle's balanced salt solution (pH 7.4; Sigma) saturated with 95% O_2 and 5% CO_2 at room temperature for 1–5 hours before mechanical dissociation. Single slices were kept in a low-Ca^{2+} buffer. Hippocampi were isolated and placed in a treatment chamber containing 1.5 mg/ml of protease XIV (Sigma) in HEPES-buffered balanced salt solution (Sigma) for 15 minutes at 35 °C, pH 7.4. Tissue was then rinsed several times in the low-Ca^{2+} buffer and triturated. The isolated cells were plated for five minutes on a glass coverslip coated with concanavalin A or poly-L-lysine (Kay and Wong, 1987a; Surmeier et al., 1992b).

2.2 Electrophysiological whole-cell recording

Recordings from hippocampal neurons were performed immediately after isolation at room temperature (22-25 °C). Patch pipettes were pulled from a thick wall borosilicate glass capillary tube on a P-97 micropipette puller (Sutter Instrument) and fire polished (final tip resistance, 2.5-5.0 MΩ). Voltage-clamp recording was obtained using an EPC-10 amplifier (HEKA) and visualized by an IX-51 inverted microscope (Olympus). Cells were held at -100 mV after achieving whole-cell voltage clamp. Voltage pulses were then delivered for 20 ms from -90 to 60 mV with 10 mV stepping at 0.5 Hz. 60-80% of the series resistance was compensated prior to recording. Voltage-clamp data were filtered at 10 kHz and digitally sampled at 200 kHz. Currents were recorded with pClamp 10 software (Axon Instruments). Data from cells with unstable or high holding currents were discarded. The whole-cell current clamp recording model was used for the recording of action potentials. A hyperpolarizating current was used to clamp the cell membrane potential at -90 mV and a 500 pA ramp depolarizing current was then input for 100 ms to generate a stimulated action potential. The amplitude and threshold values of the spike were recorded and analyzed. The external and internal electrode solutions followed Chen et al. (2005). Unless otherwise indicated, the external perfusion solution was (mM): 20 NaCl, 10 HEPES, 1 $MgCl_2$, 1 $CdCl_2$, 60 CsCl, 150 glucose, pH 7.4 (300–305 mOsm/L) with NaOH. The intracellular recording solution contained (mM): 189 N-methyl D-glucamine, 40 HEPES, 4 $MgCl_2$, 0.1 BAPTA, 1.0 NaCl, 25 phosphocreatine, 2 ATP-Na_2, 0.2 GTP, 0.1 leupeptin, pH 7.2 (270–275 mOsm/L).

To eliminate statistical error resulting from different cell membrane sizes, the current value was replaced by the current density. Current density was calculated as channel current divided by membrane capacitance. The current density-voltage curve was generated by plotting current density against step voltage.

2.3 Animals

Gene mutation was performed by means of homologous recombination as previously reported by Khasar et al. (1999). Homozygotes were obtained as follows (Chen et al., 2005). C57BL/6J mice were obtained from the Center of Laboratory Animal Science of Guangdong (Foshan, China). Wild-type female C57BL/6J mice (PKC-$\varepsilon^{+/+}$) were crossed with mutant male 129 SvJae mice (PKC-$\varepsilon^{+/-}$). The (C57BL/6J × 129 SvJae) F1 PKC-$\varepsilon^{+/-}$ mice were then intercrossed. Mice were raised and propagated in separate cages in a sterile environment. The room temperature was maintained at 24 ± 2 °C, humidity ∼ 40–60% and light cycle period 12 : 12 (light : dark). Water and food were available *ad libitum*. During the experiments, all the mice were from two to six months old. Procedures involving animals and their care were in accordance with Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council; NIH Pub. No.85-23, revised 1996), approved by Jinan Central Hospital affiliated to Shandong University (No. 2018-53-01).

Heated water-bath seizure induced mice were prepared for the FS mouse model as described previously (Zhao et al., 2016) and onset characteristics were taken as the evaluation standard. C57BL/6J mice were placed in water at 44.9 °C for five minutes and were assigned to the FS group once seizures occurred. εV1-2

treated mice were also exposed to 44.9 °C water for five minutes and are referred to as the FS + εV1-2 group. PKC-ε inhibitor εV1-2 (5 nM) (Feng et al., 2013) in FS + εV1-2 group was ICV injected into the right dorsal hippocampus 12 hours before the heated water test. Water immersion for control mice was at 37 °C for five minutes. If seizures occurred within this time, mice were immediately removed from the water. Under the FS model, mice were treated with heated water 10 times on each of two days. Hyperthermia-induced febrile seizure mice were characterized based on onset characters that included seizure latency, duration and frequency. Following a previous study (Racine, 1972), seizure frequency was classified into five stages: (1) bilateral forelimb clonus (BFC) with falling and rearing; (2) BFC and rearing; (3) unilateral forelimb clonus; (4) head nodding; and (5) facial movement. Seizure latency was measured as the time from being placed into the water to any sign of seizure onset.

2.4 Chemicals

PKC-ε inhibitor εV1-2 and its negative control were obtained from Biomol (Plymouth Meeting, PA). Recombinant IL-1β was obtained from Sigma.

2.5 Data analysis

Trace images and related data were processed for statistical analysis using Clampfit 10.0 (Axon Instruments). Numerical values are reported as mean ± SEM. In some cases, the means were tested for equality using a paired Student's *t* test. Values of $P < 0.05$ were considered significant.

3. Results

3.1 Inhibitory effect of IL-1β on Na^+ currents dependent on PKC-ε in mouse hippocampus neurons

IL-1β is known to be involved in the modulation of voltage-gated ion channels (Choi et al., 2007; Liu et al., 2006). To investigate the effects of IL-1β on Na^+ channels, here, mouse hippocampal neurons were isolated and cultured with 10 ng/ml IL-1β for 12 hours. From the results of whole-cell current recording, IL-1β inhibited the Na^+ currents in these cells (Fig. 1A). Current density-voltage curves for untreated neurons and neurons treated with 10 ng/ml IL-1β were plotted. The current density was obtained from peak current divided by membrane capacitance determined by an EPC-10 amplifier in the lock-in simulative protocol. The peak current for control cells was obtained at a step voltage of -30 mV, while IL-1β depolarized the cell membrane to -20 mV (Fig. 1B). As shown in Fig. 1C, the average peak currents from 10 cells was significantly reduced by 45.3% after IL-1β treatment (Fig. 1C). To ascertain whether IL-1β reduced Na^+ current via PKC-ε, 200 μM of εV1-2 a specific inhibitor of PKC-ε (Cang et al., 2009) was delivered to the culture medium for 30 minutes prior to IL-1β addition. As shown in Fig. 1D, εV1-2 treatment alone had no significant effect on hippocampal neurons. A cell viability assay also demonstrated that there were no alterations when using either IL-1β or εV1-2 alone, Additionally, εV1-2 significantly alleviated the inhibitory effect of IL-1β on mouse hippocampal neurons ($P < 0.01$; n = 10, Fig. 1D). Taken together, this suggested that IL-1β inhibited Na^+ currents in mouse hippocampal neurons via PKC-ε.

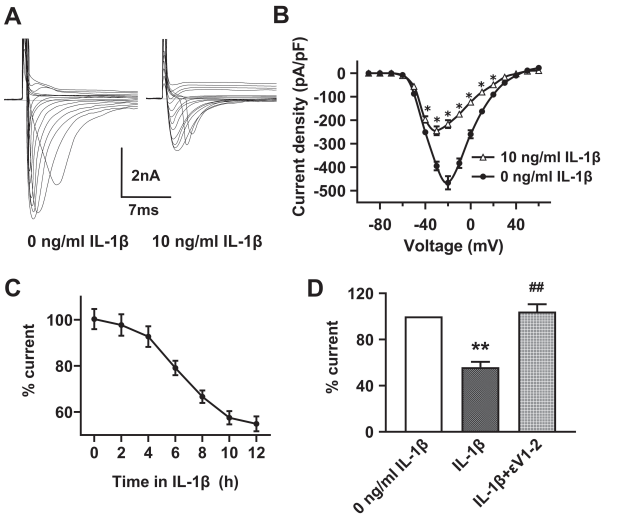

Figure 1. The inhibitory effect of IL-1β on Na^+ currents was dependent on PKC-ε in mouse hippocampal neurons. A, whole-cell current recording of the Na^+ currents in mouse hippocampal neurons (n = 10). Neurons cultured without IL-1β treatment (Left) and with 10 ng/ml of IL-1β (Right) for 12 hours. B, Comparison of current density-voltage curves between no treatment and 10 ng/ml (12 h) of IL-1β ($P < 0.05$; n = 10; Student's *t*-test), as compared with no IL-1β (*$P < 0.05$). C, Time course of the inhibitory effects of IL-1β on Na^+ current (n = 10). Data normalized to current magnitude prior to IL-1β treatment. D, Inhibitory effect of IL-1β abolished by εV1-2, an inhibitor of PKC-ε (n = 10), as compared with the no IL-1β group (**$P < 0.01$); as compared with IL-1β group ($^{\#\#}P < 0.01$, Student's *t*-test).

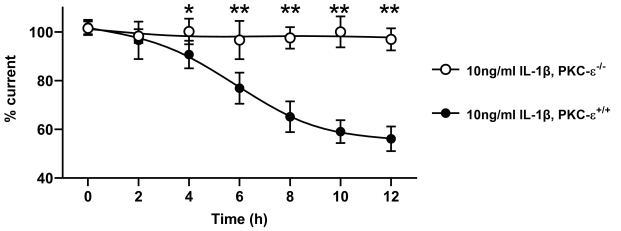

Figure 2. The inhibitory effect of IL-1β on Na^+ currents was dependent on PKC-ε *in vivo*. 10 ng/ml of IL-1β was added in the culture of the hippocampal neurons isolated from PKC-$\varepsilon^{-/-}$ mice (n = 10) and wild-type littermates PKC-$\varepsilon^{+/+}$ (n = 10). Data were normalized to the current of neurons from PKC-$\varepsilon^{+/+}$ mice not treated with IL-1β. The mean minimal current of $\varepsilon^{+/+}$ mice was inhibited by 56.1 ± 5.1%, which was significantly different from PKC-$\varepsilon^{-/-}$ mice ($P < 0.05$), as compared with 10 ng/ml of IL-1β, PKC-$\varepsilon^{-/-}$ group (*$P < 0.05$ and **$P < 0.01$).

3.2 Inhibitory effect of IL-1β on Na^+ currents dependent on PKC-ε in vivo

PKC-ε null mutant mice (PKC-$\varepsilon^{-/-}$, Khasar et al., 1999) were used to examine and analyze the role of IL-1β modulation of Na^+ currents *in vivo*. Mouse hippocampal neurons from PKC-$\varepsilon^{-/-}$ mice and wild-type littermates (PKC-$\varepsilon^{+/+}$) were isolated and cul-

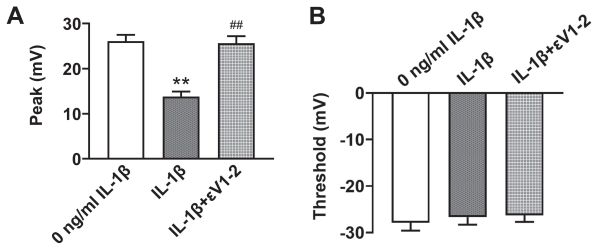

Figure 3. Effect of IL-1β and PKC-ε inhibitor εV1-2 on spike amplitude and threshold examined by current-clamp recording (n = 10). as compared with no IL-1β group ($^{**}P < 0.01$); as compared with IL-1β group ($^{\#\#}P < 0.01$, Student's t-test).

tured in 10 ng/ml of IL-1β for 12 hours. PKC-$\varepsilon^{-/-}$ mice exhibited significantly less Na^+ current reduction than PKC-$\varepsilon^{+/+}$ mice ($P < 0.05$). After 12 hours, as shown in Fig. 2, no significant reduction in Na^+ current was observed in PKC-$\varepsilon^{-/-}$ mice following IL-1β treatment as compared to the untreated mice, However, a significant reduction in Na^+ current was observed in PKC-$\varepsilon^{+/+}$ mice following IL-1β treatment as compared to untreated mice ($P < 0.05$; n = 10). Thus, in PKC-$\varepsilon^{-/-}$ mice, IL-1β showed no significant inhibitory effect on voltage-gated Na^+ current and the presence of endogenous PKC-ε aggravated the effect of reducing voltage-gated Na^+ current.

3.3 Effect of IL-1β and εV1-2 on spike amplitude and threshold in mouse hippocampal neurons

IL-1β induces repetitive firing of action potentials. To further investigate the effects of IL-1β, the action potentials of hippocampal neurons were recorded using a ramp depolarizing current. IL-1β and εV1-2 were administrated chronically to neuron cultures as described above. Results showed that neither IL-1β nor IL-1β + εV1-2 had any significant effect on the spike threshold (Fig. 3B), an important indicator of neuronal excitability. However, the peak depolarization of spikes was significantly reduced by IL-1β treatment ($P < 0.01$, n = 10), but this effect was abolished following administration of εV1-2 (n = 10, Fig. 3A). These results were consistent with the suppressing effect of current density mentioned above, which indicated that IL-1β reduced the amplitude of the action potential due to its inhibitory effect on Na^+.

3.4 Role of PKC-ε in a febrile seizure mouse model

To ascertain the role of PKC-ε in febrile seizures *in vivo*, a heated water-bath model of FS induction was employed as described previously (Zhao et al., 2016). Results demonstrated that the frequency and duration of FS in mice injected with εV1-2 were increased over the pretreatment times (Fig. 4A,C). Comparing the 10 th heat treatment to the 1st, the mean frequency was increased by 70.6% in the FS + εV1-2 group but was decreased by 11.1% in FS mice. Meanwhile, the mean FS duration was lengthened by 72.5% in the εV1-2 injection group and 14.4% in FS mice. Seizure latency of the FS group decreased slightly, and that of the FS + εV1-2 group demonstrated similar changes with no significant differences with the FS group (Fig. 4B). Compared with the 1st pretreatments in FS and FS + εV1-2 mice, the mean latency after the 10 th pretreatment was shortened by 19.0% and 18.3%, respectively. Moreover, a FS model using PKC-$\varepsilon^{-/-}$ mice by means of heat-induction was also developed following the procedures employed to generate PKC-$\varepsilon^{+/+}$ mice. After 10 treatments, the outcomes were consistent with those of the FS + εV1-2 group. These results demonstrate that PKC-ε plays a highly significant role in mouse FS.

4. Discussion

This study reports three principal findings. First, IL-1β inhibited voltage-gated sodium channels by reducing Na^+ currents in mouse hippocampal neurons and PKC-ε increased this effect. Second, peak spike depolarization was significantly reduced following treatment by IL-1β and this effect was abolished following the administration of εV1-2. However, IL-1β showed no significant impact on the spiking threshold. Third, PKC-ε reduced the susceptibility and frequency of FS onset, which might be related to the neuroprotective effect of IL-1β on hippocampus neurons.

4.1 IL-1β reduces voltage-gated Na^+ currents in mouse hippocampus neurons

Inflammatory cytokines play functional roles in neuronal excitability and, specifically, receptors for IL-1β have been found in the hippocampus at high concentration (Dubé et al., 2007). Mechanisms of FS involve alterations to molecular and functional properties, such as their effect on neurotransmitter receptors (Sanchez et al., 2001; Zhang et al., 2004) or voltage-gated ion channels (Brewster et al., 2002; Chen et al., 2001). IL-1β is known to increase seizure susceptibility by augmenting nitric oxide formation and increases neuronal excitability by direct GABA(A) receptor inhibition, increasing NMDA receptor function or inhibiting K+ efflux (Meini et al., 2000; Miller et al., 1991; Viviani et al., 2003). IL-1β rapidly inhibitsing voltage-gated Ca^{2+} channel activity, an effect that occurs within a few seconds after the addition of IL-1β to acutely dissociated hippocampus CA1 neurons (Plata-Salamán and Ffrench-Mullen, 1992, 1994) and in cultured cortical neurons (Macmanus et al., 2000). Moreover, brief exposure (five minutes) to IL-1β led to a specific (receptor-mediated) reduction of sodium current in trigeminal nociceptive neurons, while chronic treatment with IL-1β enhanced this current (Liu et al., 2006), which may be one of the mechanisms accounting for the effect of IL-1β on the nerve conduction underlying pain perception. However, in the central nervous system, the interrelations of IL-1β and Na^+ channels are unclear. In cortical neurons, IL-1β inhibits the Na^+ current via its receptor in a time- and dose-dependent manner, but does not affect voltage-dependent activation and inactivation (Zhou et al., 2011). Previous real-time PCR data shows such inhibition is caused by reducing the protein expression of Na^+ channels, which may be compensated for by other signaling pathways during long-term application of IL-1β (Zhou et al., 2006, 2011). It can be hypothesized that an effect specifically directed to the inhibition of voltage-gated Na^+ channels by IL-1β may modulate neurotoxic or neuroprotective properties related to final seizure onset. It is worth noting that only using hippocampal neurons may have limitations in fully explaining the complex pathogenesis of febrile seizures.

Neuronal injury can be both neurotoxic and neuroprotective. The dosage for IL-1β neurotoxicity varies widely from 0.05 (Viviani et al., 2003) to 100 ng/ml (Li et al., 2000) or even 100 μg/ml (Strijbos and Rothwell, 1995). This suggests the presence of mul-

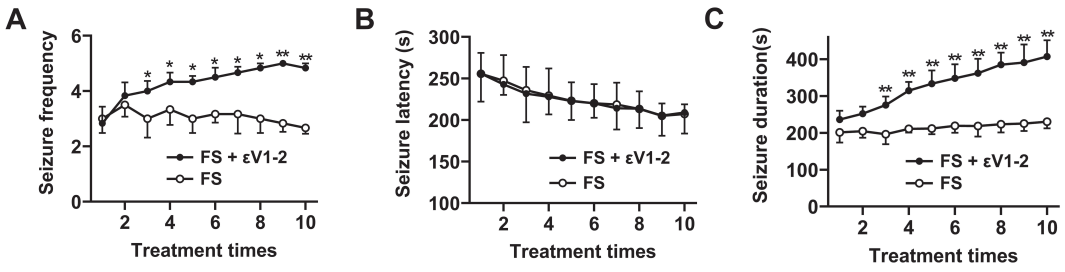

Figure 4. The susceptibility and frequency of mice (n = 10) with FS after injection of εV1-2. (A) PKC-ε inhibitor significantly reduced seizure frequency (P <0.01) and (C) duration ($P < 0.01$), (B) but had no significant impact on response latency ($P > 0.05$), as compared with the FS group (*$P < 0.05$ and **$P < 0.01$).

tiple pathway mechanisms. Moreover, the dosage for neuroprotective effects varies from 5 to 100 ng/ml (Carlson et al., 1999; Macmanus et al., 2000; Prehn, 1996), while the level of IL-1β in cerebrospinal fluid has previously been reported at 4.2 $\pm$ 1.5 pg/ml (Lahat et al., 1997). In various pathophysiological conditions, the concentration of IL-1β observed in cerebrospinal fluid can be greater than 0.1 ng/ml (Plata-Salamán and Ffrench-Mullen, 1994). As estimated previously (Zhou et al., 2006), the concentration in sensitive tissues should be increased by 1-2 magnitudes, and the maximum reaches up to 10 ng/ml. Na^+ current suppression at this dosage was found in preliminary experiments. Subsequently, mean percentages of Na^+ currents were found to be reduced to 96% (IL-1β 0.1 ng/ml), 68% (IL-1β 1 ng/ml) and 62% (IL-1β 10 ng/ml). Therefore, a 10 ng/ml concentration of IL-1β was employed in the present experiment to probe its biological effect on voltage-gated Na^+ channels. During the first four hours of the application of 10 ng/ml IL-1β, no significant changes of Na^+ currents were obtained (Fig. 1C). This may be a consequence of a reduced number of Na^+ channels in hippocampal neuron membranes, which was consistent with the modulation of their expression in both alveolar epithelial cells (Roux et al., 2005) and cortical neurons (Zhou et al., 2011)

4.2 PKC-ε potentiates inhibitory effect of IL-1β on Na^+ current and action potential amplitude

εV1-2 has been used as an inhibitor of PKC-ε (Zhou et al., 2011). In Zhou's study, the inhibitor of PKC (chelerythrine; 1 μM) and p38 MAPK (SB 203580; 1 μM) were applied 30 minutes prior to IL-1β. Results indicated that the inhibitory effect of IL-1β on Na^+ current in cortical neurons was dependent on PKC and then p38 MAPK. Consequently, it can be hypothesized that Na^+ channels are regulated through a mechanism similar to that existing in other neurons, the main reason being that the PKC-ε isoform is highly expressed in hippocampal neurons (C Tanaka and Nishizuka, 1994; Naik et al., 2000) and Na^+ channels and PKC-ε are widely coexpressed (Chen et al., 2005). The present study explored the roles of PKC-ε via selective inhibition by a PKC-ε inhibitor εV1-2 and targeted gene deletion.

In mouse hippocampal neurons, the Na^+ current was significantly reduced with extracellular treatment by IL-1β ($P < 0.01$), while the application of IL-1β + εV1-2 abrogated the inhibitory effect of IL-1β with no significant change in current magnitude when compared to control. Thus, PKC-ε contributes to the inhibitory effect of IL-1β. This was consistent with a previously reported effect of PKC on IL-1β (Zhou et al., 2011). Additionally, the endogenous PKC-ε existing in normal neurons does not inhibit Na^+ currents in the absence of an increased concentration of IL-1β. This indicates that the absence of PKC-ε does not impact Na^+ currents even if IL-1β is increased and PKC-ε may be capable of co-opting this mechanism in pathological cells. Further an effect of PKC-ε on IL-1β was identified in PKC-$\varepsilon^{-/-}$ mouse neurons, while the inhibition of Na^+ current was similar to results obtained from experiments using PKC-ε inhibitor. As previously reported, PKC-ε is required for selective regulation of cardiac Na^+ channels, which may work by interacting with Na^+ channel proteins (Xiao et al., 2001). PKC-ε knock-out mice show no change in Na^+ currents in response to PKC-ε activation in hippocampal neurons, suggesting PKC-ε as the PKC isozyme that serves as a regulator for voltage-gated Na^+ channels (Barnett et al., 2007; Chen et al., 2005).

4.3 PKC-ε decreases susceptibility and frequency of febrile seizure onset

It has been reported that IL-1β shows proconvulsive effects before FS onset. IL-1β degrades electrographic activity and prolongs behavioral seizure duration in rodent epilepsy models (Vezzani et al., 1999, 2002). Seizure-like behavioral changes were significantly reduced following inhibition of IL-1β production by the treatment of an inhibitor of a IL-1β-converting enzyme (Donnelly et al., 2001). Moreover, administration of an extra-high dose (116 ng/2 μl) of IL-1β is sufficient to generate seizures in the absence of increased brain temperature (Dubé et al., 2005). Endogenous IL-1β contributes to hyperexcitability and seizures generated by fever and hyperthermia in mice, but this is improved by injecting IL-1β six minutes prior to seizure induction (Dubé et al., 2005).

Further, the chronic effect of IL-1β may be different to that in acute situations. Acute administration of IL-1β to trigeminal nociceptive neurons inhibits Na^+ currents, whereas its chronic effect potentiates Na^+ currents (Liu et al., 2006). Thus, the effect of PKC-ε on IL-1β may be determined by the type of application. To investigate the properties of PKC-ε during chronic IL-1β exposure, in the present study, the FS model mice were generated by heated-water treatment for two hours following each 12 hour application of εV1-2. The seizure frequency and duration following exposure to εV1-2 were exacerbated and prolonged significantly, possibly resulting from abrogation of the neuroprotective effect of IL-1β by εV1-2. Therefore, PKC-ε may be involved in an IL-1β neuroprotective effect in FS. After ICV injection of εV1-2,

the occurrence rate and characteristics of seizures were consistent with the FS group before the first heated water treatment (data not shown). That is to say, application of PKC-ε inhibitor cannot be the only factor that affects FS onset. Thus, endogenous IL-1β and PKC-ε both exert functions acting on FS via fever or hyperthermia.

Taken together, IL-1β inhibits the Na^+ currents in mouse hippocampal neurons and the PKC-ε contributes the effect of IL-1β. Targeted gene deletion experiments identified the effects of IL-1β and PKC-ε. IL-1β reduced the amplitude of action potentials resulting from its inhibitory effect on the Na^+ channels, which may decrease the release of presynaptic transmitters of neuroexcitability which in turn is neuroprotective for excitotoxicity in hippocampal neurons. Moreover, PKC-ε reduces the susceptibility and frequency of FS onset via the neuroprotective effect of IL-1β in hippocampal neurons. IL-1β's effect on the Na^+ channel and the role of PKC-ε in FS has been demonstrated. However, effect of K^+ currents or other calcium currents have not been ruled out in the present study.

Ethics approval and consent to participate

The animal use protocol listed below has been reviewed and approved by Jinan Central Hospital affiliated to Shandong University (No.2018-53-01). Approval No. IACUC DB-16-025.

Authors' contributions

JLW and HYZ designed the research study. FFX and YZ performed the research. XC and PPZ provided help and advice on the experiments.

Acknowledgment

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Conflict of interest

The authors declare no competing interests.

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