

Pilocarpine-induced status epilepticus alters hippocampal PKC expression in mice

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We investigated the protein expression of different protein kinase C (PKC) isoforms (PKC α , PKC β 1, PKC β 2, PKC γ , PKC δ , PKC ϵ , PKC η and PKC ζ) in the hippocampus of normal control mice and progressive changes in PKC isoforms expression during and after pilocarpine induced status epilepticus (PISE). We showed the reduced expression of PKC δ , PKC η and PKC ζ in interneurons in the CA1 area and in the hilus of the dentate gyrus during or after PISE. Increased expression of PKC α and PKC β 1 was demonstrated in the stratum pyramidale of CA3 area, and PKC ϵ was up-regulated in the stratum lucidum of the CA3 area during or after PISE. Our results suggest that hippocampal PKC isoforms may play different roles in seizure generation, and be targets for development of anti-convulsive drugs.

Key words: epilepsy, hippocampus, status epilepticus, protein kinase C, PKC isoforms

INTRODUCTION

Recent studies have suggested that protein kinase C (PKC) isoforms may be involved in epileptogenesis (Ono et al. 1994, Guglielmetti et al. 1997, Tang et al. 2004, Cuellar et al. 2005, Niimura et al. 2005, Silva et al. 2007, Liu et al. 2008, Terunuma et al. 2008). However, the exact roles of each PKC isoform in epileptogenesis remain elusive (Ono et al. 1994, Guglielmetti et al. 1997, Liu et al. 2008) due to inconsistent results from previous studies. Furthermore, in many of the previous studies, western blot (McNamara et al. 1999), biochemical analysis (Daigen et al. 1991, Kohira et al. 1992, Ono et al. 1994) and *in situ* hybridization (Guglielmetti et al. 1997) were used in different animal models of epilepsy or seizure. The former two approaches could not locate PKC isoforms in different hippocampal neurons and their processes, since mRNA changes of PKC isoforms from *in situ* hybridization study may not reflect changes of the protein expression.

In the present study in the mouse pilocarpine model of status epilepticus (SE) and temporal lobe epilepsy

(TLE), we investigated the protein expression of PKC α , PKC β 1, PKC δ , PKC ϵ , PKC η and PKC ζ in different cell types and lamina of the hippocampus at different time points during and after pilocarpine-induced status epilepticus (PISE), in order to correlate these protein changes to the disease pathogenesis. Similar study for PKC β 2 and PKC γ was not done as the results for induced expression of the two isoforms has been reported in our previous study (Liu et al. 2008).

METHODS

Pilocarpine treatment

B29 male and female mice weighing 25–30 g were used for the study according to our established procedure. Mice were given a single subcutaneous injection of methyl-scopolamine nitrate (1 mg/kg) 30 min before the injection of either saline in the control or pilocarpine in the experimental groups. In the latter group, mice received a single i.p. injection of 300 mg/kg pilocarpine and experienced acute SE. In this experiment, 9% animals died during or after SE induction. Therefore, they were not included in the quantitative study. All experiments were approved by the Tan Tock Seng

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Hospital – National Neuroscience Institute Institutional Animal Care and Use Committee. In the handling and care of all animals, the guidelines of the NIH for animal research were strictly followed. Efforts were made throughout the study to minimize animal suffering and to use the minimum number of animals.

Video camera and electroencephalography (EEG) monitoring

To monitor EEG of the freely moving mice, a transmitter (TSE, Bad Homburg, Germany) was fixed on the electrode socket by plug connection with wires attached to the skull of mice by 2 screws (2.3 mm posterior and 2 mm lateral to bregma) 3 days before pilocarpine induction. The EEG signals were telemetrically received via HF receiver which passed the signals to the computer. The duration of EEG monitoring was 24 hours starting from 30 minutes before injection of methyl-scopolamine nitrate. Special telemetry interfaces built in the computer decoded and processed the signals from the receiver and then transferred them to the personal computer. Signals were then read by the TSE TeleSys data acquisition and analysis program. Video camera recording was done simultaneously to correlate behavioral changes to EEG data.

Immunohistochemical study of the expression of PKC isoforms

A total of 36 mice was used for immunohistochemical study. Six mice were killed at each of the following survival intervals, i.e., at 30 min, 2 h during PISE, at 1 day, 1 week and 2 months after PISE. Six mice with saline instead of pilocarpine injection were sacrificed for the control group. Following deep anesthesia with chloral hydrate (0.4 g/kg), the mice were perfused transcardially with 10 ml of saline followed by 100 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4) for 10 min. After perfusion, the brain of each mouse was removed, and kept overnight in 30% sucrose in 0.1 M PB. Coronal sections at 40 μ m thickness were cut in a cryostat (HM505E, Microm, Zeiss, Germany). Serial sections were transferred to different wells of a 24-well tissue culture dish for controls, PKC α , PKC β 1, PKC β 2, PKC γ , PKC δ , PKC ϵ , PKC η , PKC ζ immunohistochemical reactions.

For immunocytochemical study, free-floating sections were treated with 4% normal goat serum for 2 h at room temperature. All sections were then incubated

with primary rabbit antibodies for PKC ϵ , PKC η , PKC ζ (1:400; Sigma, St Louis, USA), PKC δ (1:500; Santa Cruz Biotechnology, Inc, CA, USA) and mouse antibodies for PKC α (1:200), PKC β 1 (1:100), PKC β 2 (1:200), PKC γ (1:200; Santa Cruz Biotechnology Inc., CA, USA) in 0.1 M Tris-buffered saline (TBS) containing 0.1% Triton X-100 (TBS-TX, pH 7.6) overnight. After incubation, sections were washed in TBS-TX and placed for 2 h in biotinylated anti-rabbit (for PKC δ , PKC ϵ , PKC η and PKC ζ) or anti-mouse (PKC α , PKC β 1, PKC β 2 and PKC γ) secondary antibodies. After three washes in TBS-TX, the sections were placed in avidin-biotin complex (ABC) reagent (Vector Laboratories Inc., Burlingame, CA, USA) in TBS-TX for 2 h. They were then washed in 0.1 M Tris buffer (TB, pH 7.6), incubated in a solution of 0.012% H₂O₂ and 0.05% 3,3'-diaminobenzidine (Sigma, St Louis, USA) in TB for 3-5 min, mounted, and coverslipped. For negative control, the primary antibodies were omitted.

Cell counting and statistical analysis

Data analysis was done by using i-SOLUTION/LITE (Micro Optics Systems, Singapore). Four to five sections of the temporal part of the dorsal hippocampus from each mouse were used for densitometry or cell counting. For densitometry, the intensity of immunopositive staining was measured and indicated as a grey value ranging from 255 to 0. The higher the grey value is, the less the immunostaining in the sections. The immunopositive neuronal profiles of PKC isoforms in the strata oriens, radiatum, and lacunosum moleculare of the CA1 area, and in the hilus of the dentate gyrus were counted and the area of each respective layer was measured. Cell density was then indicated as the number of cells per square millimeter. Quantitative data were indicated as mean value \pm standard deviation, and then subjected to statistical analysis by one-way ANOVA followed by Student–Newman–Keuls *post hoc* multiple comparisons.

In SE mice at 1 week or 2 months after PISE, only a few survived neurons existed in the CA1 area and in the hilus of the dentate gyrus, which was consistent with our previous studies. This made impossible to differentiate different layers of the hippocampus. The changes of PKC isoforms at these two time points may be related to cell loss instead of down-regulation of protein expression. Therefore, the quantitative analysis of protein expression in different layers of CA1 area and in the hilus of the dentate gyrus after PISE was not done.

RESULTS

Behavioral and EEG monitoring

In experimental groups, pilocarpine-induced behavioral changes include hypoactivity, tremor, head bobbing, myoclonic movements of the limbs, recurrent myoclonic convulsions with rearing, falling, and status epilepticus. The onset of continuous epileptic spiking activity, which was considered to be a marker for the onset of status epilepticus, was found at 28.72 ± 10.84 min after pilocarpine injection. The continuous epileptic spiking activity (>0.5 Hz) lasted for about 7.23 ± 1.59 h.

Protein expression of PKC isoforms in the hippocampus of control and experimental mice during and after pilocarpine-induced status epilepticus

PKC α

In the control mice (Fig. 1A), moderate PKC α immunopositive staining was observed in the strata oriens and radiatum of CA1 area, whereas strong PKC α immunopositive staining was demonstrated in the two layers in CA3 area. In the dentate gyrus, weak PKC α immunopositive staining was shown in the stratum molecular and in the hilus (Fig. 1A). In CA3a area, some pyramidal neurons were also PKC α immunopositive (arrows in Fig. 2A). At 1 day after PISE (Fig. 2D), the PKC α immunopositive staining in the stratum pyramidale of CA3b increased significantly compared with that of the control (Fig. 2A; $p < 0.01$, Fig. 2G). At 30 min (Fig. 2B) and 2 hrs (Fig. 2C) during pilocarpine induce SE, the distribution of PKC α immunopositive staining in the CA3b was similar to control (Fig. 2A; $p > 0.05$, Fig. 2G). At 1 week (Fig. 2E) and 2 months (Fig. 2F) after PISE, PKC α immunopositive staining was mainly demonstrated in surviving neurons in the stratum pyramidale of CA3a area (arrows in Fig. 2E, F).

PKC β 1

In the control mice (Fig. 1B), PKC β 1 exhibited a similar distribution pattern to PKC α . Moderate PKC β 1 immunopositive staining was observed in the strata oriens and radiatum in CA1 areas, whereas strong PKC β 1 immunopositive staining was demonstrated in

those layers of CA3 area. In the dentate gyrus, weak PKC β 1 immunopositive staining was evenly distributed in the stratum molecular and hilus (Fig. 1B). In CA3a area, some pyramidal neurons were also PKC β 1 immunopositive (arrows in Fig. 3A). At 1 day after PISE (Fig. 3D), PKC β 1 immunopositive staining in the stratum pyramidale of CA3b increased significantly when compared to the control (Fig. 3A; $p < 0.01$, Fig. 3G). At 30 min (Fig. 3B) and 2 hrs (Fig. 3C) during PISE, PKC β 1 immunopositive staining in CA3b was similar to the control (Fig. 3A; $p > 0.05$, Fig. 3G). At 1 week (Fig. 3E) and 2 months (Fig. 3F) after PISE, PKC β 1 immunopositive staining was mainly present in surviving neurons in the stratum pyramidale of CA3a area (Fig. 3E, F).

PKC β 2

In the control mice (Fig. 1C), PKC β 2 was strongly expressed in the strata oriens, radiatum and lacunosum molecular of CA1 area. Almost no immunopositive staining was found in other areas of hippocampus (Fig. 1C, 4A). In the stratum pyramidale of CA3 area, the immunopositive staining of PKC β 2 was up-regulated significantly at 1 day after PISE (Fig. 4D) compared to the control (Fig. 4A; $p < 0.01$, Fig. 4G), whereas no significant changes were found among the control mice and mice at 30 min (Fig. 4B) and 2 hrs (Fig. 4C) during PISE ($p > 0.05$, Fig. 4G). At 1 week (Fig. 4E) and 2 months (Fig. 4F) after PISE, the expression of PKC β 2 in reactive astrocytes was induced.

PKC γ

In the control mice (Fig. 1D), PKC γ was strongly expressed in the strata oriens, radiatum and lacunosum molecular of CA1 area. Moderate PKC γ immunostaining was also shown in CA3 area. Evenly stained immunopositive band was observed in the inner molecular layer of the dentate gyrus (Fig. 1D, 5A). PKC γ immunopositive staining decreased significantly in the hilus of the dentate gyrus at 2 hrs during PISE (Fig. 5C; $p < 0.05$, Fig. 5H), 1 day (Fig. 5D; $p < 0.05$, Fig. 5H), 1 week (Fig. 5E; $p < 0.05$, Fig. 5H) and 2 months (Fig. 5F; $p < 0.05$, Fig. 5H) after PISE, and in the inner molecular layer of the dentate gyrus at 1 day (Fig. 5D; $p < 0.05$, Fig. 5G), 1 week (Fig. 5E; $p < 0.01$, Fig. 5G) and 2 months (Fig. 5F; $p < 0.01$, Fig. 5G) after PISE when compared to the control (Fig. 5A; intensity in hilus: 228.3 ± 2.3 , intensity in the inner molecular layer: 220.3 ± 7.6).

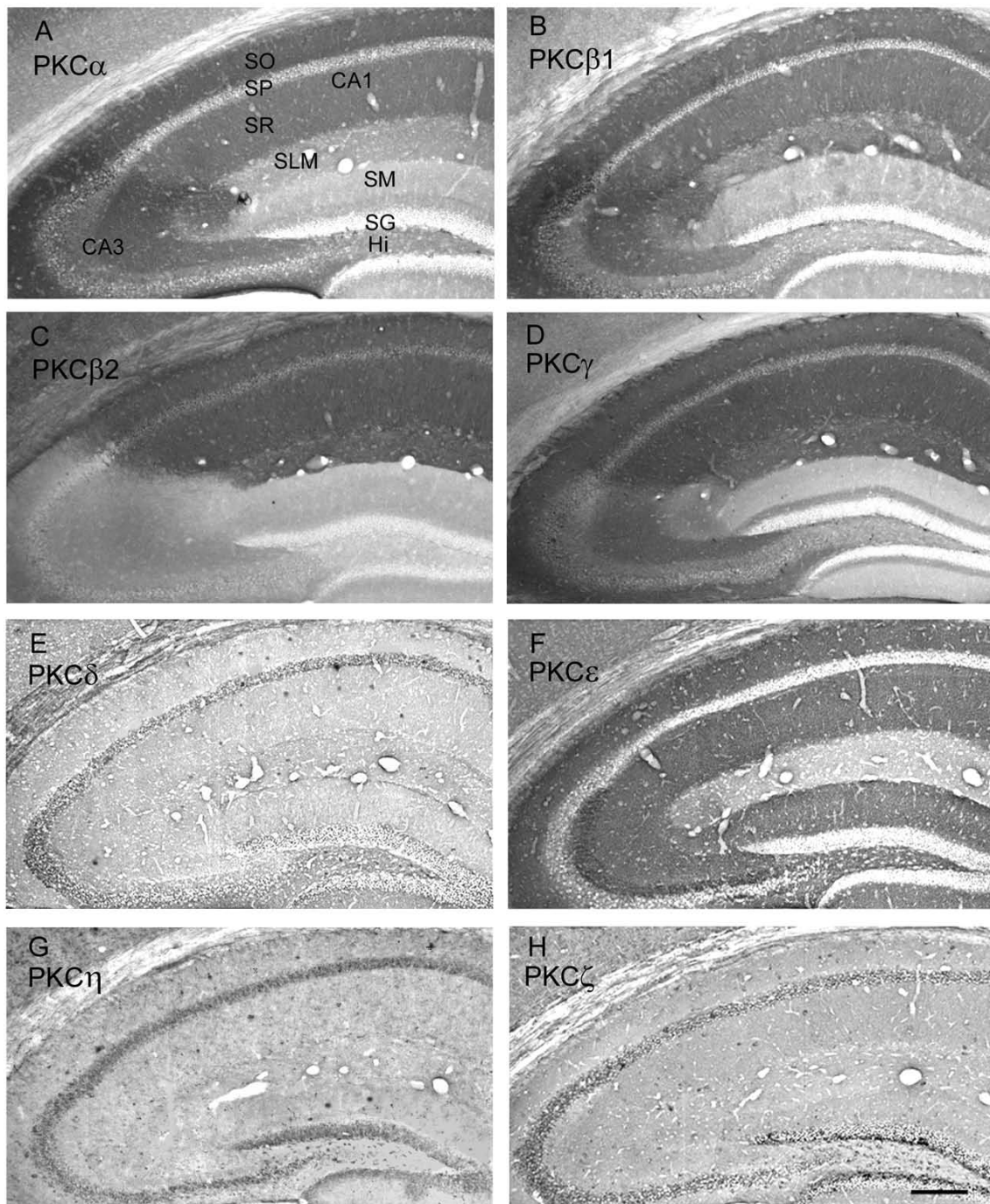


Fig. 1. Immunohistochemistry shows the protein expression pattern of PKC α (A), PKC β 1 (B), PKC β 2 (C), PKC γ (D), PKC δ (E), PKC ϵ (F), PKC η (G) and PKC ζ (H) in the hippocampus of control mice. PKC α (A), PKC β 1 (B), PKC β 2 (C), PKC γ (D), and PKC ϵ (F) are evenly distributed in CA1 and CA3 areas, some pyramidal neurons are PKC α (A) and PKC β 1 (B) immunopositive. PKC ϵ (F) immunopositive product is localized in the stratum lucidum. In the dentate gyrus, PKC γ (D) immunopositive band appears in the inner molecular layer, however, PKC ϵ (F) immunopositive staining is present in the entire molecular layer. PKC δ (E), PKC η (G) and PKC ζ (H) immunopositive neurons are located in the stratum pyramidale of CA1-3 areas and in the stratum granulosum of the dentate gyrus. Some interneurons in CA1-3 areas and in the hilus of dentate gyrus are also PKC δ (E), PKC η (G) and PKC ζ (H) positive.

SO - stratum oriens, SP - stratum pyramidale, SR - stratum radiatum, SLM - stratum lacunosum moleculare, SM - stratum moleculare, SG - stratum granulosum, Hi - hilus of the dentate gyrus. Scale bar = 200 μ m in H applies to A-G.

PKC δ

In the control mice, PKC δ (Fig. 1E, 6A) immunopositive neurons located in the stratum pyramidale of CA1-3 and in the stratum granulosum of the dentate gyrus. Some interneurons at the border between the stratum oriens and alveus (O/A border), in the strata oriens, radiatum and lacunosum moleculare of CA1-3 areas, and in the hilus of the dentate gyrus were also PKC δ immunopositive (Fig. 1E, 6A). In the stratum radiatum of CA1 area, significant reduction of PKC δ immunopositive neurons was observed at 30 min (Fig. 6B; $p < 0.01$, Fig. 6G), 2 hrs (Fig. 6C; $p < 0.01$, Fig. 6G) during PISE and 1 day (Fig. 6D; $p < 0.01$, Fig. 6G) after PISE when compared to the control (Fig. 6A). At 1 week (Fig. 6E) and 2 months (Fig. 6F) after PISE, the expression of PKC δ was induced in reactive astrocytes.

PKC ϵ

In the control mice (Fig. 1F), moderate to strong PKC ϵ immunoreactivity was demonstrated in the strata oriens and radiatum of CA1-3 area. In the molec-

ular layer of the dentate gyrus (Fig. 1F), strong PKC ϵ immunopositive staining was found. PKC ϵ immunopositive staining was also localized in the stratum lucidum of CA3 area and in the hilus of the dentate gyrus where mossy fibers and terminals were located (Fig. 1F, 7A; arrows in Fig. 7A). In the stratum pyramidale of CA1-3 area and stratum granulosum of the dentate gyrus, there was almost no PKC ϵ immunostaining (Fig. 1F, 7A).

PKC ϵ immunopositive staining in the stratum lucidum of CA3 area or in mossy fibers and terminals increased obviously at 1 week after PISE (Fig. 7E) when compared to the control (Fig. 7A; $p < 0.05$, Fig. 7G), whereas there were not significant differences among control and other experimental groups, i.e., 30 min (Fig. 7B) and 2 hrs (Fig. 7C) during PISE, and 1 day (Fig. 7D) and 2 months (Fig. 7F) (intensity: 196.8 ± 7.8) after PISE ($p > 0.05$, Fig. 7G).

PKC η

In the control mice (Fig. 1G, 8A, E), PKC η immunopositive neurons were located in the stratum pyramidale of

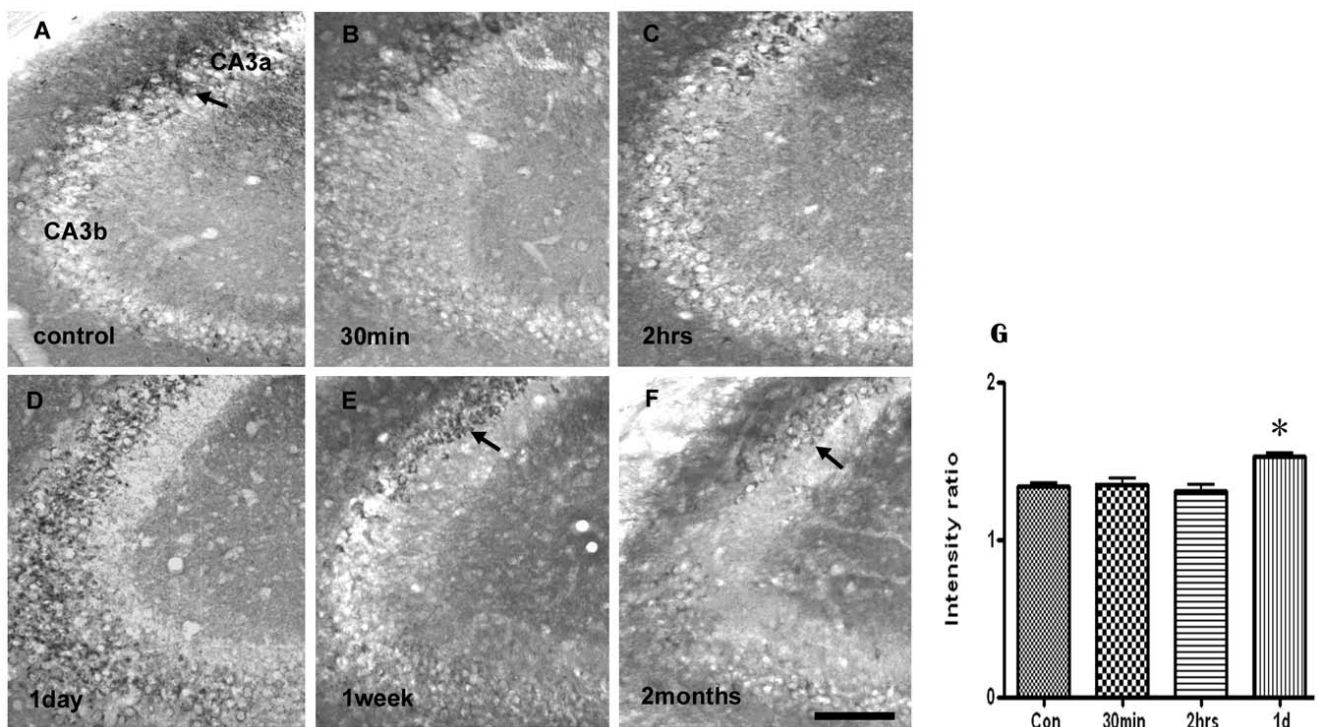


Fig. 2. At 1 day after PISE (D), PKC α immunopositive staining in stratum pyramidale of CA3b increases significantly compared to the control (A), ($*p < 0.01$, G). No significant changes are found between control, 30 min (B) and 2 hrs (C) during PISE. PKC α is still expressed in surviving neurons at 1 week (E) and 2 months (F) after PISE. Scale bar = 100 μ m in F applies to A-E. Histogram (G) shows the progressive changes of PKC α immunoreactivity in the stratum pyramidal of CA3b area. $*p < 0.01$, when compared to control.

CA1-3 areas and in the stratum granulosum of the dentate gyrus. Some interneurons in the strata oriens, radiatum and lacunosum moleculare of CA1-3 areas and in the hilus of the dentate gyrus were also PKC η immunopositive.

Compared to the control (Fig. 8E), the number of PKC η immunopositive neurons decreased significantly in the strata oriens ($p<0.01$, Fig. 8K), radiatum ($p<0.01$, Fig. 8L) and lacunosum moleculare ($p<0.05$ or $p<0.01$, respectively, Fig. 8M) at both 30 min (Fig. 8F) and 2 hrs (Fig. 8G) during PISE, but no change was observed at 1 day after PISE (Fig. 8H).

In the hilus of dentate gyrus, a significant reduction of PKC η immunopositive neurons was found at 30 min (Fig. 8B; $p<0.01$, Fig. 8N), 2 hrs (Fig. 8C; $p<0.01$, Fig. 8N) during PISE and 1 day after PISE (Fig. 8D; $p<0.01$, Fig. 8N) when compared to the control mice (Fig. 8A). One week (Fig. 8I) and 2 months (Fig. 8J) after PISE, PKC η was induced in reactive astrocytes.

PKC ζ

In the control mice (Fig. 1H, 9A), PKC ζ immunopositive neurons were located in the stratum pyramidale

of CA1-3 areas and in the stratum granulosum of the dentate gyrus. Some interneurons in the strata oriens, radiatum and lacunosum moleculare of CA1-3 areas and in the hilus of the dentate gyrus were also PKC ζ immunopositive.

In the hilus of dentate gyrus, a significant reduction of PKC ζ immunopositive neurons was found at 30 min (Fig. 9B; $p<0.01$, Fig. 9G), 2 hrs (Fig. 9C; $p<0.01$, Fig. 9G) during PISE and 1 day after PISE (Fig. 9D; $p<0.01$, Fig. 9G) when compared to the control mice (Fig. 9A). At 1 week (Fig. 9E) and 2 months (Fig. 9F) after PISE, PKC ζ was induced in reactive astrocytes.

In sections with the primary antibodies omitted, no immunostaining was observed.

DISCUSSION

Main findings

In the present study, we compared the expression of different PKC isoforms in the hippocampus of the normal B29 mice and experimental ones during and after PISE. In the hippocampus of the control mice, each

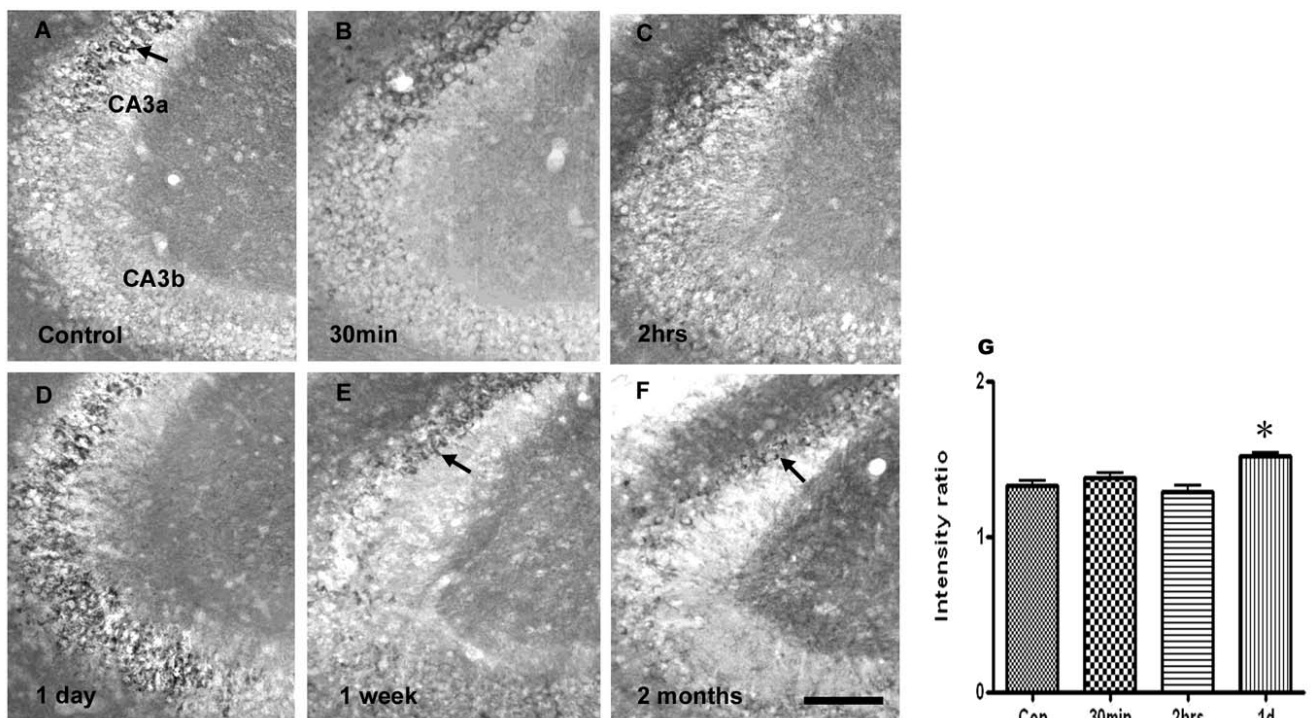


Fig. 3. PKC β 1 immunopositive staining in the stratum pyramidale of CA3b area increases significantly at 1 day after PISE (D) when compared to the control (A), ($*p<0.01$, G). There is no change of PKC β 1 immunopositive staining in the stratum pyramidale of CA3b among control, 30 min (B) and 2 hrs (C) during PISE. PKC β 1 immunopositive staining is also shown in surviving neurons at 1 week (E) and 2 months (F) after PISE. Scale bar = 100 μ m in F applies to A-E. Histogram (G) shows the progressive changes of PKC β 1 immunoreactivity in the stratum pyramidale of CA3b area. $*p<0.01$, when compared to control.

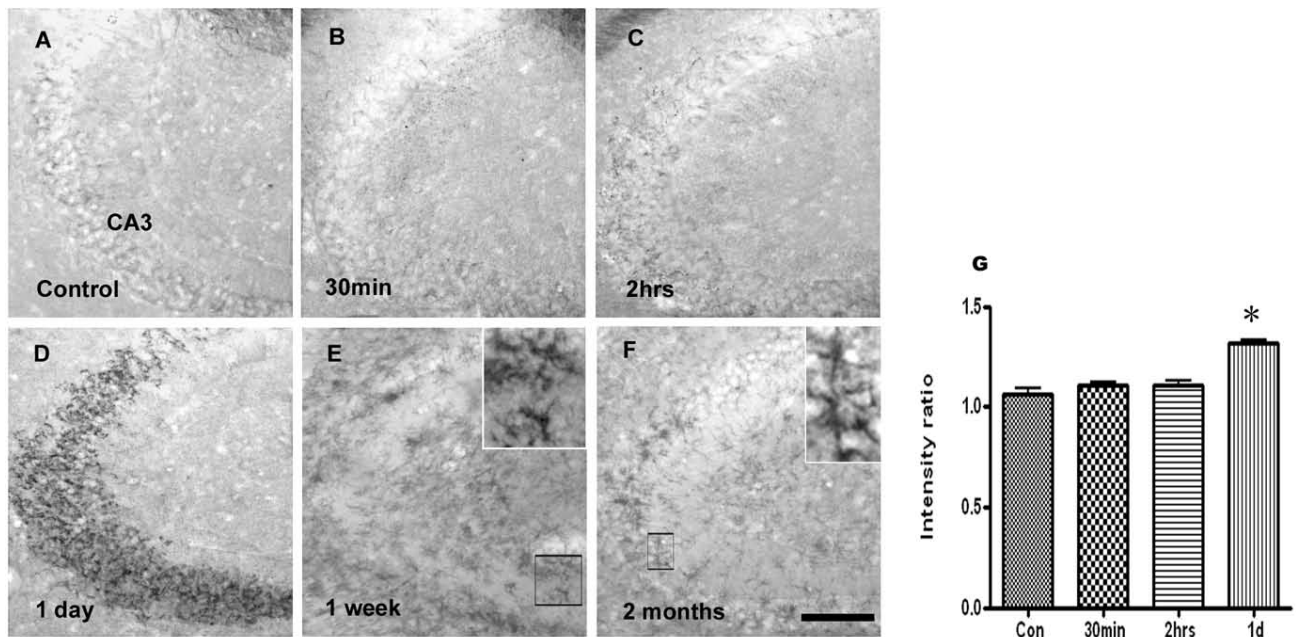


Fig. 4. When compared to the control (A), PKCβ2 immunopositive staining increases significantly in the stratum pyramidale of CA3 area at 1 day after PISE (D), ($*p<0.01$, G). There is no significant difference between the control and 30 min (B) and 2 hrs (C) during PISE ($p>0.05$, G). At 1 week (E) and 2 months (F) after PISE, PKCβ2 is induced in reactive astrocytes. Scale bar = 100 μm in F applies to A-E. Histogram (G) shows the progressive changes of PKCβ2 immunoreactivity in the stratum pyramidale of CA3 area. $*p<0.01$, when compared to control.

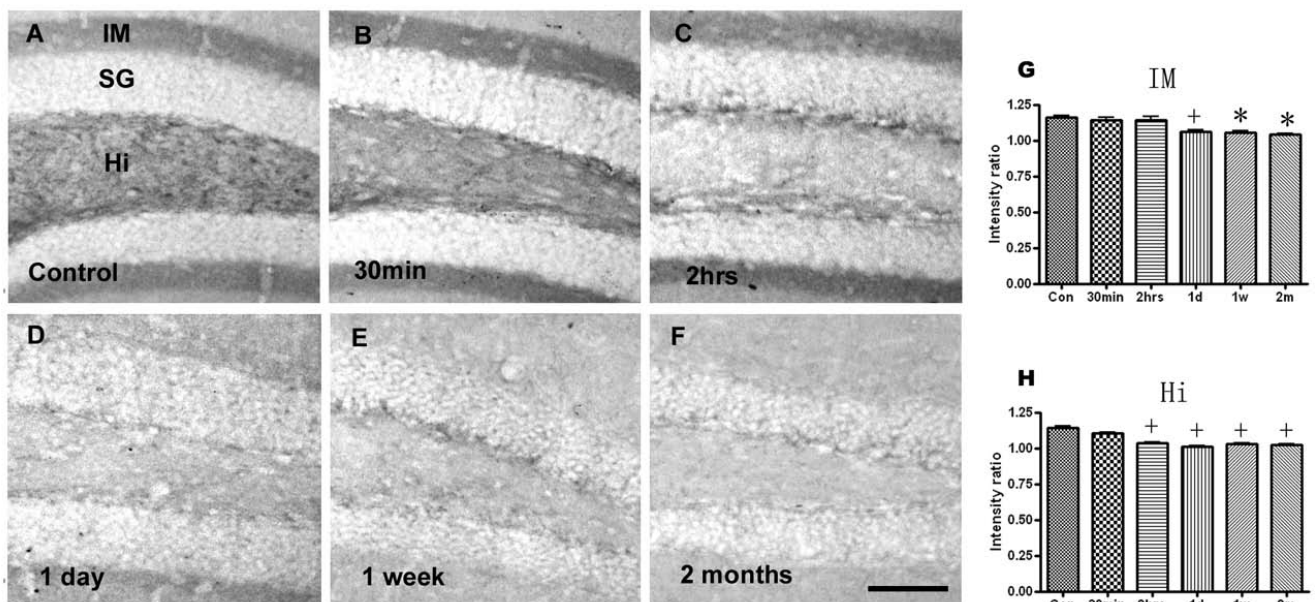


Fig. 5. When compared to the control (A), PKCγ immunopositive staining in the hilus of the dentate gyrus decreases significantly at 2 hrs (C), 1 day (D), 1 week (E) and 2 months (F) during or after PISE ($+p<0.05$, H). In the inner molecular layer of the dentate gyrus, PKCγ immunopositive staining decreases significantly at 1 day (D), 1 week (E) and 2 months (F) after PISE when compared to the control (A), ($p<0.01$ or $p<0.05$, G). Scale bar = 100 μm in F applies to A-E. Histograms (G, H) show the progressive changes of PKCγ immunoreactivity in the inner molecular layer and in the hilus of the dentate gyrus. $*p<0.01$, $+p<0.05$, when compared to the control.

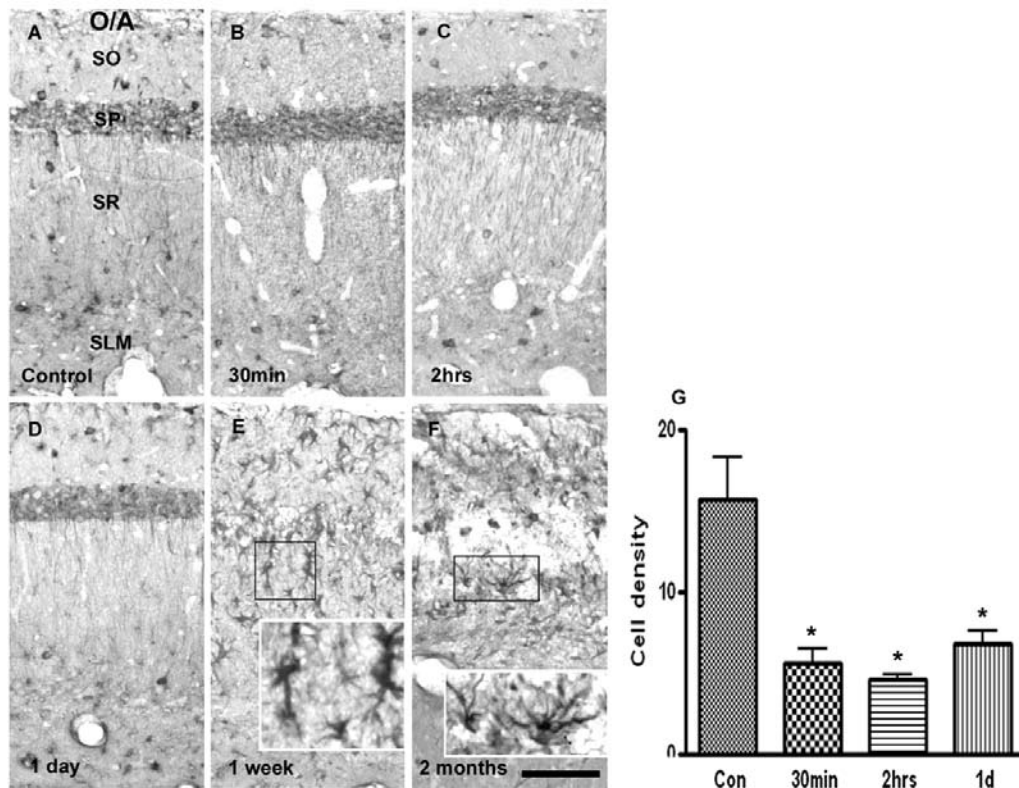


Fig. 6. In the stratum radiatum of CA1 area, the number of PKC δ immunopositive neurons decreases significantly at 30 min (B), 2 hrs (C) during PISE and 1 day after PISE (D) compared to the control (A), (* p <0.01, G). At 1 week (E) and 2 months (F) after PISE, the PKC δ is expressed in reactive astrocytes. Scale bar = 100 μ m in F applies to A-E. Histogram (G) shows the progressive changes of PKC δ immunoreactivity in the stratum radiatum of CA1 area. * p <0.01, when compared to control.

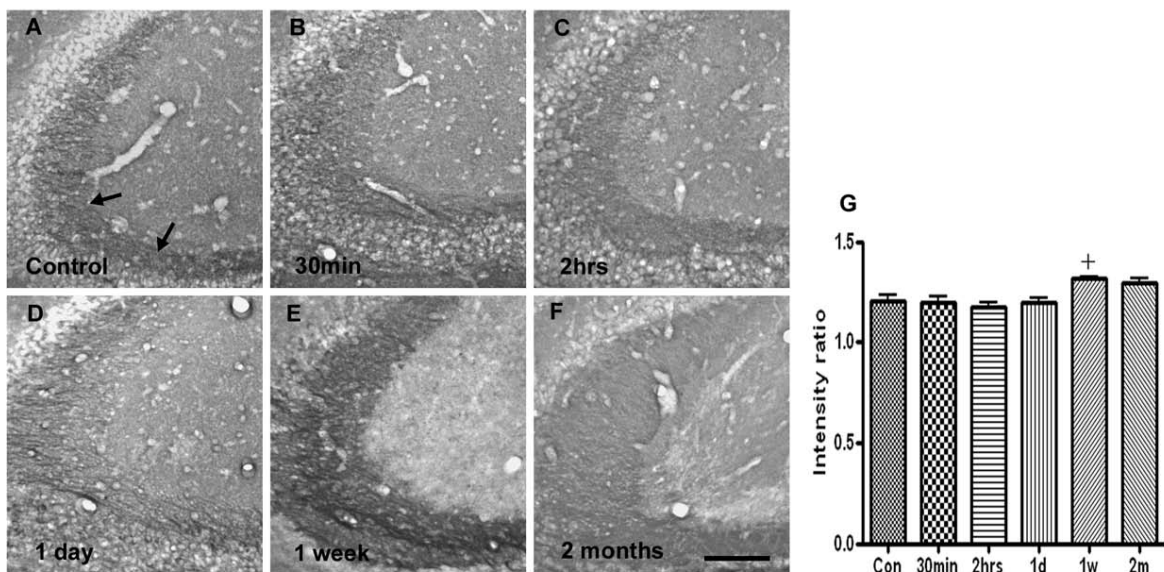


Fig. 7. PKC ϵ immunopositive staining in the stratum lucidum of CA3 area increases significantly at 1 week after PISE (E) compared to the control (A), (+ p <0.05, G). No significant change is demonstrated between control (A), 30 min (B), 2 hrs (C), 1 day (D) and 2 months (F) during or after PISE. Scale bar = 100 μ m in F applies to A-E. Histogram (G) shows the progressive changes of PKC ϵ immunoreactivity in the stratum lucidum of CA3 area. + p <0.05, when compared to control.

PKC isoform exhibited unique expression profile. In the experimental mice, we showed significant reduction of PKC δ and PKC η immunopositive interneurons in CA1 area at early stages during PISE, up-regulation of PKC α , PKC β 1 in CA3b area, PKC β 2 in stratum pyramidale of CA3 area at 1 day after PISE, up-regulation of PKC ϵ in stratum lucidum of CA3 area at 1 week after PISE. The progressive reduction of PKC η and PKC ζ immunopositive neurons in the hilus, and PKC γ immunopositive product in the inner molecular layer of the dentate gyrus was also demonstrated. At 1 week or 2 months after PISE, the expressions of PKC β 2, PKC δ , PKC η and PKC ζ in reactive astrocytes was induced.

The present study also indicated the differences of PKC expression between the rat (Tang et al. 2004) and mouse model of status epilepticus and temporal lobe epilepsy. For instance, PKC β 2 and PKC γ immunopositive staining was much stronger in CA1 than CA3 area in the mouse, whereas the immunopositive product of these two isoforms were evenly distributed in strata oriens and radiatum of CA area in the rat.

Progressive changes of PKC isoforms in hippocampal neurons during and after PISE

PKC activation is a critical part of intracellular cascade triggered by metabotropic receptors. Cellular effects of PKC activation include reduced spike accommodation, antagonism of a voltage-sensitive chloride current (Madison et al. 1986), suppression of the potassium current (Grabauskas et al. 2006), activating Na⁺ current (Bich-Hoai et al. 2010) and accelerated calcium clearance (Usachev et al. 2006) from the cytoplasm. Most of these effects would be expected to enhance intrinsic cellular excitability (Fuortes et al. 2008). In the present study, down-regulation of PKC δ and PKC η in CA1 interneurons at the early stage during PISE could result in the initiation of neurodegenerative process and lower activity of these GABAergic neurons, leading to the disinhibition of pyramidal neurons, and subsequent SE. In previous study, Ono and coauthors (1994) showed the unchanged activities of PKC α in hippocampus at 1 and 4 weeks after the last generalized kindled seizure. In the present study, up-regulation of PKC α and PKC β 1 was demonstrated in the stratum pyramidale of CA3b area at 1 day after PISE, it may be related to the hyperactivity of CA3 pyramidal neurons (Ono et al. 1994). Up-regulation of PKC β 2 in CA3 area in the present study is consistent with the pre-

vious study showing PKC β subtypes, it may play an important role in the enduring seizure susceptibility

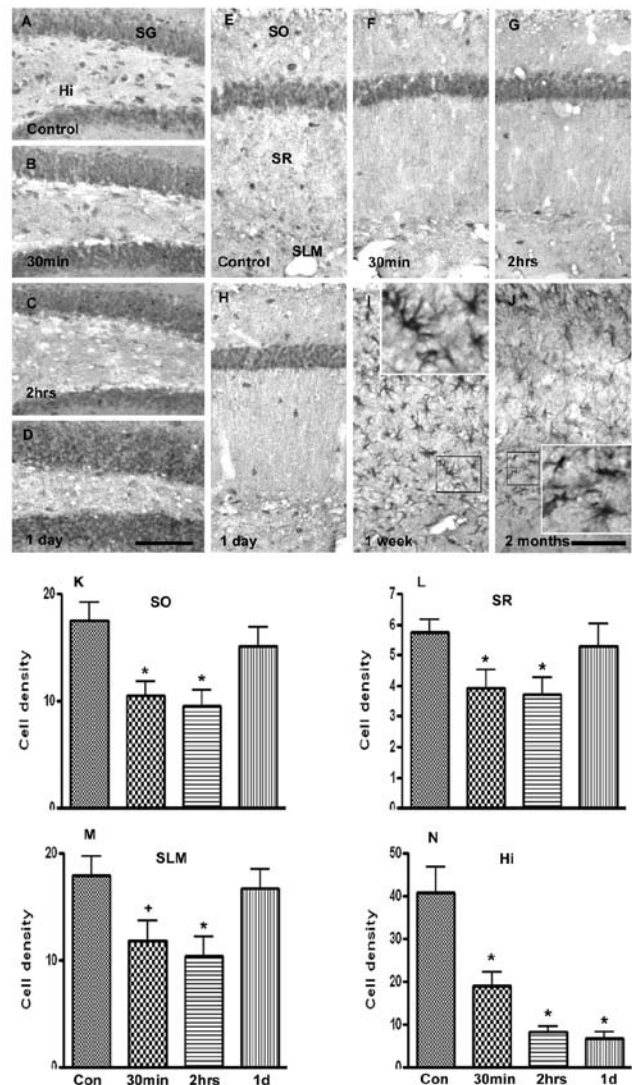


Fig. 8. In the strata oriens (K), radiatum (L) and lacunosum moleculare (M) of CA1 area, the number of PKC η immunopositive neurons decreases significantly at both 30 min (F) and 2 hrs (G) during PISE compared to the control (E) respectively (* p <0.01, K, L, M). No obvious change is observed at 1 day after PISE (H). In CA1 area, PKC η is induced in the reactive astrocytes at 1 week (I) and 2 months (J) after PISE. In the hilus of the dentate gyrus, the number of PKC η immunopositive neurons decreases progressively at 30 min (B and N), 2 hrs (C and N) and 1 day (D and N) during or after PISE when compared to the control (A), (* p <0.01, N). Scale bar = 100 μ m in J applies to E-I, 100 μ m in D applies to A-C. Histograms show the progressive changes of PKC η immunoreactivity in the different layers of CA1 area, respectively. * p <0.01, when compared to control.

associated with kindling (Ono et al. 1994). Down-regulation of PKC η and PKC ζ in the hilus of the dentate gyrus in the present study may initiate neurodegenerative changes and subsequently delayed loss of hilar neurons at 1 day after PISE. Loss of mossy cells could be linked to the reduction of PKC γ expression in the inner molecular layer of dentate gyrus. In kainic acid model, immunostaining showed up-regulation of PKC ϵ in mossy fibers at 1 week and 2 months after SE (Guglielmetti et al. 1997). It was supported by our present study showing an increased expression of PKC ϵ immunostaining in mossy fibers and/ or their terminals in the pilocarpine

model. Functionally, PKC ϵ could form catalytically active complexes with actin and these complexes are closely associated with enhancement of glutamate release from nerve terminals (Coffey et al. 1993, Prekeris et al. 1996, Guglielmetti et al. 1997). We therefore speculate that increased PKC ϵ in mossy fibers and their terminals may specifically influence glutamate release in CA3 area of the hippocampus and play a role in epileptogenesis at the latent period after PISE.

Reactive glial cells in CA1 area are supposed to release neurotrophic factors that support axonal sprouting and new synaptogenesis, leading to the develop-

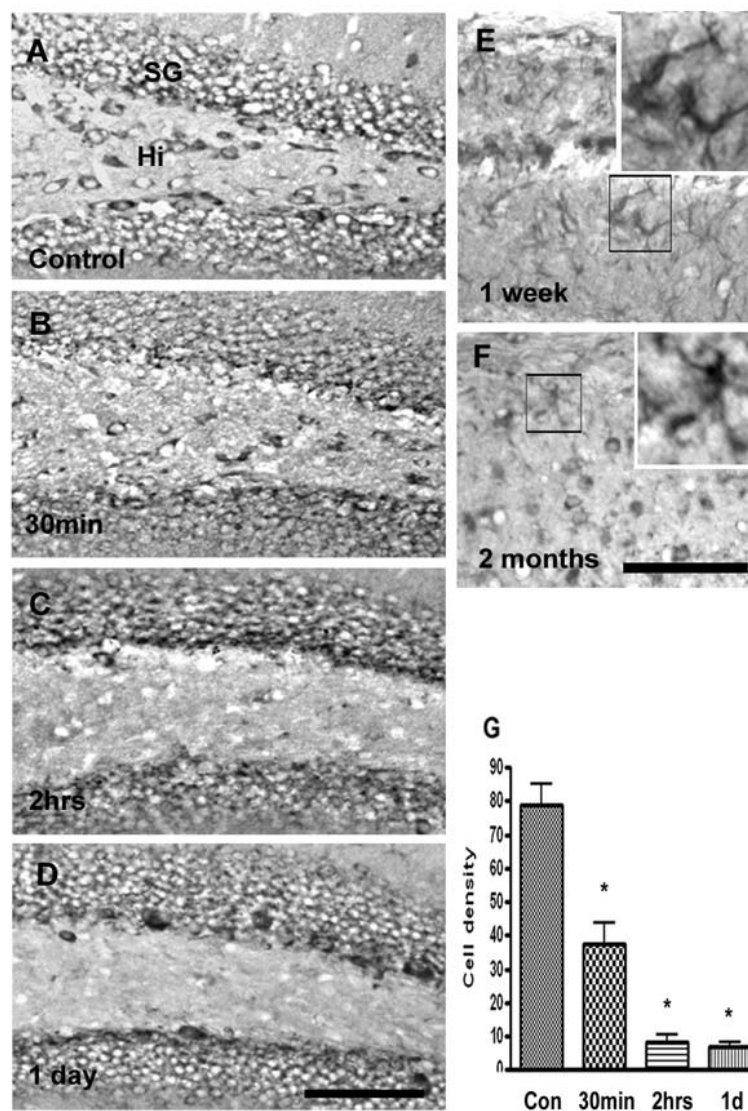


Fig. 9. In the hilus of the dentate gyrus, the number of PKC ζ immunopositive neurons decreases progressively at 30 min (B), 2 hrs (C) and 1 day (D) during or after PISE when compared to the control (A), (* p <0.01, G). At 1 week (E) and 2 months (F) after PISE, PKC ζ was induced in reactive astrocytes. Scale bar = 100 μ m in D applies to A-C, 100 μ m in F applies to E. Histogram (G) shows the progressive changes of PKC ζ immunoreactivity in the hilus. * p <0.01, when compared to control.

Table I

The intensity of PKC isoform immunoreactivity and density of PKC isoform stained cells in the hippocampus of control and PISE mice.

	Control	30min	2hrs	1d	1w	2m
PKCα						
CA3b intensity	191.5 \pm 7.4	189.7 \pm 13.9	195.2 \pm 12.1	167.3 \pm 4.8*		
PKCβ1						
CA3b intensity	191.8 \pm 8.0	186.5 \pm 12.3	198.2 \pm 12.0	167.8 \pm 5.0*		
PKCβ2						
SP.CA3 intensity	240.7 \pm 15.8	230.8 \pm 7.6	230.3 \pm 10.5	194.2 \pm 7.4*		
PKCγ						
IM intensity	220.3 \pm 7.6	223.8 \pm 8.3	224.7 \pm 14.2	240.7 \pm 5.8 ⁺	241.8 \pm 5.3*	245.5 \pm 4.6*
Hi intensity	228.3 \pm 2.3	231.7 \pm 5.9	246.5 \pm 4.8 ⁺	252.2 \pm 2.5 ⁺	247.3 \pm 3.8 ⁺	249.2 \pm 4.9 ⁺
PKCδ						
SR.CA1 cell density	15.8 \pm 6.3	5.6 \pm 2.3*	4.6 \pm 0.8*	6.9 \pm 1.9*		
PKCϵ						
SL.CA3 intensity	211.7 \pm 10.7	214.0 \pm 13.2	217.3 \pm 10.8	213.7 \pm 9.8	193.8 \pm 4.3 ⁺	196.8 \pm 7.8
PKCη						
SO.CA1 cell density	17.5 \pm 4.3	10.5 \pm 3.2*	9.5 \pm 3.7*	15.1 \pm 4.5		
SR.CA1 cell density	5.8 \pm 0.9	3.9 \pm 1.5*	3.7 \pm 1.3*	5.3 \pm 1.8		
SLM. CA1 cell density	17.9 \pm 4.4	11.8 \pm 4.5*	10.5 \pm 4.2*	16.8 \pm 4.4		
Hi cell density	40.9 \pm 14.4	18.9 \pm 8.3*	8.3 \pm 2.9*	6.7 \pm 3.7*		
PKCζ						
Hi cell density	78.7 \pm 16.0	37.5 \pm 15.1*	8.2 \pm 5.2*	6.6 \pm 3.5*		

SP.CA3 - stratum pyramidale of CA3, IM - inner molecular layer of DG, Hi - hilus, SR.CA1 - stratum radiatum of CA1, SL.CA3 - stratum lucidum of CA3, SO.CA1 - stratum oriens of CA1, SLM CA1 - stratum lacunosum moleculare of CA1; * p <0.01, + p <0.05, when compared to control.

ment of a chronic hyperexcitability (Represa et al. 1995, Crespel et al. 2002). Induced expression of PKC β 2, PKC δ , PKC η or PKC ζ in glial cells at 1 week and 2 months after PISE therefore suggests that these isoforms may be involved in epileptogenesis.

In the present study, the PKC positive neuronal profiles were defined only by neuroanatomical and morphological characteristics, and claimed reactive astrocytes signal came from cells morphologically resembling astrocytes. The quantitative study of protein expression of PKC isoforms has been explored only in dorsal hippocampus instead of the whole hippocampus. In addition, down-regulation of PKC isoforms in CA1 area at acute stages during and after PISE (30 min, 2 hrs and 1 day), and in the hilus at 30 min or 2 hrs during PISE, were not caused by neuronal loss, which had been shown clearly in our previous study (Liu et al. 2007).

CONCLUSION

The protein expression of different PKC isoforms was changed during and after PISE. Down-regulation of PKC δ , PKC η and PKC ζ in interneurons at the early stages during PISE may lead to the disinhibition of pyramidal neurons, while up-regulation PKC α , PKC β 1 and PKC ϵ in CA3 area may be involved in the hyperactivity of CA3 pyramidal neurons. In addition, induced expression of PKC isoforms in glial cells at chronic stages after PISE suggests that it may play a important role in epileptogenesis.

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