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Status epilepticus alters hippocampal PKAβ and PKAγ expression in mice☆

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ABSTRACT

Objectives: To investigate the localization and progressive changes of cyclic-AMP dependent protein kinase (cPKA) in the mouse hippocampus at acute stages during and after pilocarpine induced status epilepticus.

Methods: Pilocarpine induced status epilepticus mice were sacrificed 30 min, 2 h or 1 day after the start of a ~7 h lasting status as assessed by video-electroencephalography. Brains were processed for quantitative immunohistochemistry of hippocampal cPKAβ and cPKAγ, and immunohistochemical co-localization of cPKAβ and cPKAγ with calbindin (CB), calretinin (CR), and parvalbumin (PV).

Results: Based on anatomical and morphological assessment, cPKAβ was primarily expressed by principal cells and cPKAγ by interneurons. In CA1, cPKAβ co-localized with 76% of CB, 41% of CR, and 95% of PV-immunopositive cells, while cPKAγ co-localized with 50% of CB, 29% of CR, and 80% of PV-immunopositive cells. Upon induction of status epilepticus, cPKAβ expression was transiently reduced in CA1, whereas cPKAγ expression was sustainably reduced.

Conclusion: cPKA may play an important role in neuronal hyperexcitability, death and epileptogenesis during and after pilocarpine induced status epilepticus.

1. Introduction

The mammalian protein kinase A (PKA) family consists of four regulatory and three catalytic subunits, and each subunit is encoded by a unique gene. Cyclic-AMP dependent protein kinase (cPKA), a serine–threonine kinase, is involved in a wide range of cellular functions such as metabolism, transport, cell cycle, and gene regulation. Furthermore, cPKA is involved in controlling hippocampal neuronal activity,[1–4] and has been reported to participate in maintaining epileptic activity and inducing status epilepticus.[5–10] For instance, in tottering mice[5] or genetically prone to audiogenic seizures (GPAS) rats,[6] the abnormal elevation of PKA activity has been demonstrated to contribute to a functional impairment of $GABA_A$ receptor, and be involved in mechanisms of genetic predisposition to spontaneous seizures. In status epilepticus (SE) model, Niimura et al.[7] observed that phosphorylation of NR1 by PKA was depressed relative to controls immediately following SE and transiently increased above control values upon the termination of SE. Vázquez-López et al.[8] suggested that acute picrotoxin-induced seizures occurred without an increase in PKA activity in the hippocampus, but reduced PKA-mediated phosphorylation protected against picrotoxin seizures by increasing the inhibitory potential of $GABA_A$ receptors. In hippocampal interneurons, it has been shown that H2 histamine receptor activation negatively modulates outward currents through $Kv_{3.2}$-containing potassium channels by a mechanism involving PKA phosphorylation and PKA phosphorylation of $Kv_{3.2}$ lowered the maximum firing frequency of inhibitory neurons, which in turn negatively modulated high-frequency population oscillations recorded in principal cell layers.[11] However, so far, there is no study to describe detailed localization of cPKA in the hippocampus, particularly in different subtypes of calcium binding protein (CBP) (calbindin, CB; calretinin, CR; parvalbumin, PV) immunopositive interneurons. Furthermore, the progressive changes of cPKA in the hippocampus at acute stages, i.e., within 1 day, during and after pilocarpine induced status epilepticus are also unknown. Therefore, the present study was carried out to address these issues in order to provide clues for better understanding the roles that cPKA might play in inducing or maintaining status epilepticus, and in neurodegenerative changes at acute stages during and after pilocarpine induced status epilepticus.

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2. Materials and methods

2.1. 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 two 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 h starting from 30 min 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, so to correlate behavioral changes to EEG data.

2.2. Pilocarpine treatment

B29 male and female mice weighing 25–30 g were used for the study according to our established procedure.[12,13] 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, the mice received a single i.p. injection of 300 mg/kg pilocarpine and experienced acute SE. In this experiment, 9% animals died during and after induction. Therefore, they were not included in the quantitative study. All experiments were approved by the Tan Tock Seng 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.

2.3. Immunohistochemical study of the expression of cPKAβ and cPKAγ

A total of 24 mice was used for immunohistochemical study. Six mice were killed at each of the survival intervals after beginning of the status epilepticus, i.e. at 30 min, 2 h during pilocarpine induced status epilepticus, and at 1 day after pilocarpine induced status epilepticus. Six mice with saline instead of pilocarpine injection were killed at 30 min, 2 h and 1 day for the control group. Following deep anaesthesia with chloral hydrate (0.4 g/kg), the mice were perfused transcardially with 100 ml of saline initially, followed by 100 ml of 4% paraformaldehyde in 0.1 M PB (phosphate buffer, pH 7.4) for 15 min. After perfusion, the brain of each mouse was removed, and kept overnight in 30% sucrose in 0.1 M phosphate buffer. Coronal sections at 40 μm thickness were cut in a cryostat (HM505E, Microm; Zeiss, Oberkochen, Germany). Serial sections were transferred to different wells of a 24-well tissue culture dish for controls and PKA immunohistochemical reaction. For immunohistochemical study, free-floating sections were treated with 2% lyophilized bovine serum albumin in TBS–TX (Tris-buffered saline with 1‰ Triton X-100, PH 7.6) (for cPKAβ and cPKAγ) for 2 h at room temperature. All sections were then washed in TBS–TX and placed overnight at room temperature in the primary goat antibodies against cPKAβ (1:500 in TBS–TX) and cPKAγ (1:1000 in TBS–TX) (Santa Cruz Biotechnology, CA, USA). After incubation, sections were washed in TBS–TX and placed for 2 h in biotinylated anti-goat IgG secondary antibodies (1:400 in TBS–TX). After three washes in TBS–TX, the sections were placed in avidin–biotin complex (ABC) reagent (Vector Laboratories, Burlingame, CA, USA, 1:100 in TBS–TX) for 2 h. They were then washed in 0.1 M Tris buffer (TB, pH 7.6) and reacted in a solution of 0.012% H_2O_2 and 0.05% 3,3′-diaminobenzidine (Sigma, St. Louis, MO, USA) in TB for 3–5 min and washed in TB, mounted, dehydrated and covered. In the negative control section, the primary antibodies were omitted.

2.4. Double-labeling immunofluoresence microscopy

Double-labeling immunofluoresence microscopy was carried out to co-localize cPKAβ and cPKAγ with different subtypes of calcium binding protein (calbindin, calretinin and parvalbumin from Chemicon International Inc., CA, USA) in hippocampal neurons. For this study, tissue sections from six control mice were washed in 0.1 M TBS–TX and placed overnight in primary antibodies (cPKAβ: 1:500, cPKAγ: 1:1000, calbindin: 1:2000, calretinin: 1:1500, parvalbumin: 1:1500). After incubation, sections were washed in TBS–TX and placed for 4–6 h in Cy3-conjugated donkey anti-goat IgG (1:200, Chemicon International Inc., CA, USA), followed by incubation with FITC-conjugated goat anti-mouse IgG (1:200, Chemicon International Inc., CA, USA) for 4–5 h. The sections were then mounted, dried, and covered with FluorSave™ Reagent (Calbiochem, San Diego, CA, USA) to retard fading, and examined with an Olympus Fluoview FV500 (Olympus Corporation, Tokyo, Japan) confocal laser scanning biological microscope.

2.5. Cell counting and statistical analysis

Cell counting was carried out using the Image-Pro Plus system (Media Cybernetics, Silver Spring, MD, USA). The cPKAβ and cPKAγ immunopositive neuronal profiles (defined by neuroanatomical and morphological characteristics without evidence of co-localization with neuronal marker) in the strata oriens, radiatum, lacunosum moleculare, granulosum and in the hilus of the dentate gyrus were counted and indicated as a number per square millimeter. In the stratum pyramidale of CA1, the intensity value was measured and was normalized to the intensity value of corpus callosum. 3–5 sections (the intersection distance is 40 μm × 8 μm = 320 μm) of the dorsal hippocampus from each animal were used for cell counting or intensity measurement. Immunofluorescent neurons were counted under the confocal microscope, and the ratios of the number of double-labeled neurons to all the calbindin-, calretinin- or parvalbumin labeled neurons were obtained. For every section (3–5 sections for every animal), all calbindin, calretinin or parvalbumin immunopositive cells in the respective areas were counted. To avoid the false immunopositive cell counting, double-labeled cells were confirmed under high magnification (400×) with two different filters. Each cell was also tilted at two vertical planes in order to confirm that the yellow color was produced by the red and green labeling in the same cell instead of two overlapped cells with different colors. All the data were then subjected to statistical analysis by one-way ANOVA followed by Student–Newman–Keuls posthoc multiple comparisons.

3. Results

3.1. Behavioral and EEG monitoring

In the mouse model, pilocarpine-induced behavioral changes include hypoactivity, tremor, head bobbing, and myoclonic movements of the limbs progressing to 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.

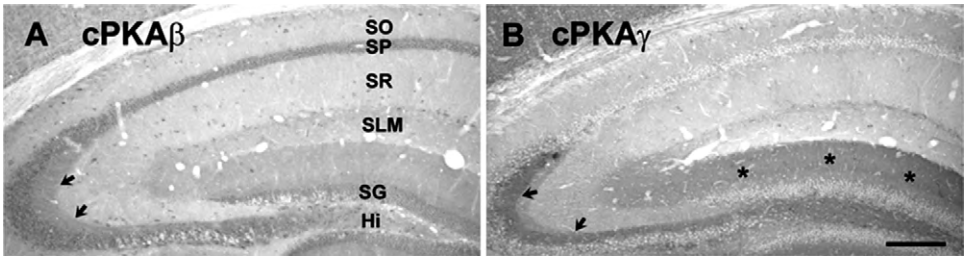

Fig. 1. Immunocytochemical staining shows cPKAβ (A) and cPKAγ (B) immunopositive product in the hippocampus of the control mice. Moderate cPKAβ immunopositive product is localized in the stratum pyramidale (SP) of CA1-3 areas and in the stratum granulosum (SG) of the dentate gyrus. Weak cPKAβ immunopositive product is located in the stratum lucidum of CA3 area (arrows). Moderately to strongly stained cPKAγ (B) immunopositive product is localized in the hilus and molecular layer (asterisks) of the dentate gyrus and in the stratum lucidum (arrows) of CA3 area. Scale bar = 200 μm, also applies to (A).

3.2. *cPKAβ and cPKAγ in the hippocampus of the control mice*

In mouse hippocampus, moderately to strongly stained cPKAβ (Figs. 1A and 2A) or cPKAγ (Figs. 1B and 2E) immunopositive neurons were found in the strata oriens, radiatum and lacunosum moleculare of the CA1-3 area, and in the hilus of the dentate gyrus (Fig. 3A for cPKAβ and E for cPKAγ). cPKAβ immunopositive neurons were also demonstrated in the stratum pyramidale of CA1-3 area and the stratum granulosum of the dentate gyrus (Fig. 1A, 2A and 3A). Moderately stained cPKAβ immunopositive product was also localized in the stratum lucidum of CA3 area where mossy fibers and terminals were located (Fig. 1A). Strong cPKAγ immunopositive product appeared in the molecular layer and hilus of the dentate gyrus and in the stratum lucidum of CA3 area (Fig. 1B).

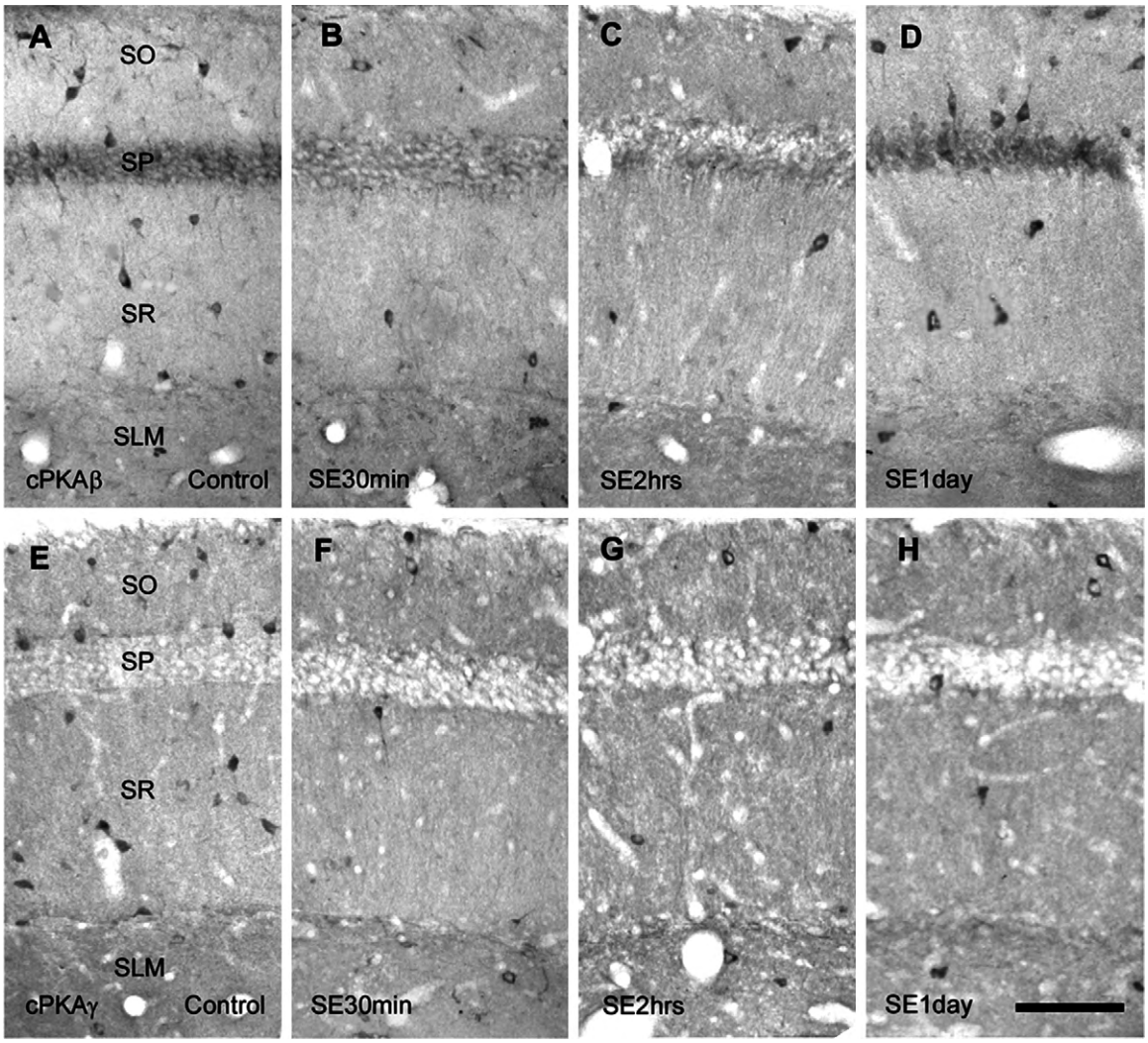

Fig. 2. cPKAβ (A–D) immunopositive product in the stratum pyramiale of the CA1 area decreases significantly at 30 min (B) and 2 h during pilocarpine induced status epilepticus (C) compared to the control (A), but returns back at 1 day after pilocarpine induced status epilepticus (D). In different strata of CA1 area, the number of cPKAβ immunopositive neurons decreases at 30 min (B) and 2 h during pilocarpine induced status epilepticus (C), while the number of cPKAγ (E–H) immunopositive neurons decreases at 30 min (F), 2 h during (G) and 1 day after (H) pilocarpine induced status epilepticus compared to the control (E). (H) Scale bar = 100 μm, also applies to (A–G).

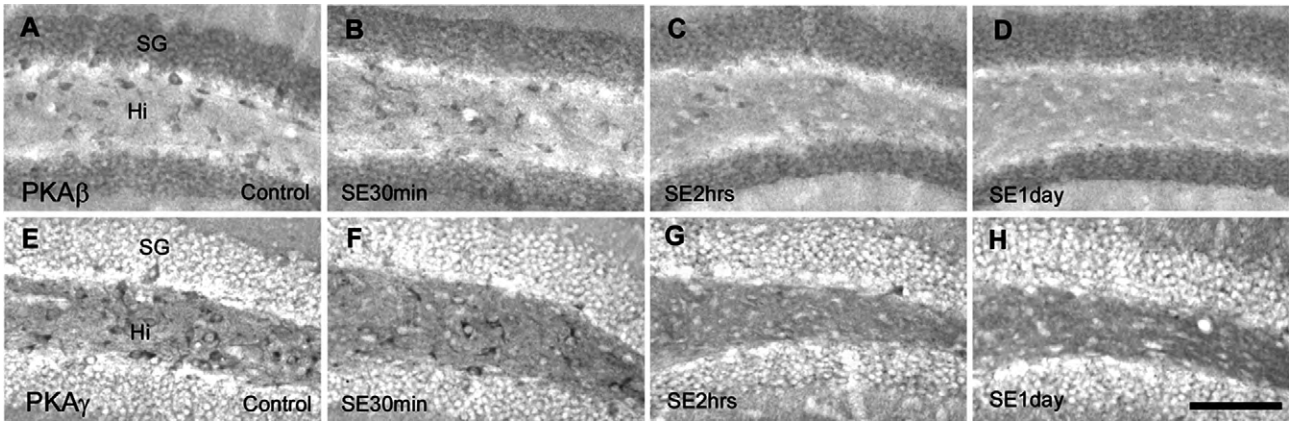

Fig. 3. The number of cPKAβ (A–D) and cPKAγ (E–H) immunopositive neurons decreases progressively in the hilus of the dentate gyrus from 30 min during (cPKAβ: B; cPKAγ: F) to 1 day after (cPKAβ: D; cPKAγ: H) pilocarpine induced status epilepticus. (H) Scale bar = 200 μm, also applies to (A–G).

3.3. *Protein expression of cPKAβ and cPKAγ in the hippocampus of experimental mice at acute stages during and after pilocarpine induced status epilepticus*

3.3.1. *CA1 area*

3.3.1.1. Stratum pyramidale. Compared to the control mice (Fig. 2A), the number of cPKAβ immunopositive neurons reduced significantly at both 30 min (Fig. 2B) ($P < 0.05$, Fig. 4D) and 2 h during pilocarpine induced status epilepticus (Fig. 2C) ($P < 0.01$, Fig. 4D), but returned back at 1 day after pilocarpine induced status epilepticus (Fig. 2D, $P > 0.05$, Fig. 4D). At all the time points, cPKAγ immunopositive product was almost undetectable in the stratum pyramidale of CA1 area (Fig. 2E–H).

3.3.1.2. Strata oriens and radiatum. At 30 min (Fig. 2B) and 2 h (Fig. 2C) during pilocarpine induced status epilepticus, the number of cPKAβ immunopositive neurons also decreased significantly compared with the control (Fig. 2A) ($P < 0.01$, Fig. 4A and B), but it was similar to the control at 1 day after pilocarpine induced status epilepticus (Fig. 2D) ($P > 0.05$, Fig. 4A and B). In the two layers, the number of cPKAγ immunopositive neurons reduced significantly at 30 min (Fig. 2F), 2 h during (Fig. 2G) and 1 day after pilocarpine induced status epilepticus (Fig. 2H) compared to the control (Fig. 2E) ($P < 0.05$, Fig. 4A and B). However, no significant difference was shown for PKAγ immunopositive neurons among 30 min, 2 h during and 1 day after pilocarpine induced status epilepticus ($P > 0.05$, Fig. 4A and B).

3.3.1.3. Stratum lacunosum moleculare. At all the time points during (30 min: Fig. 2B and F; 2 h: Fig. 2C and G) and after (1 day: Fig. 2D and H) pilocarpine induced status epilepticus, the number of both cPKAβ (Fig. 2B–D) and cPKAγ (Fig. 2F–H) immunopositive neurons in the stratum lacunosum moleculare

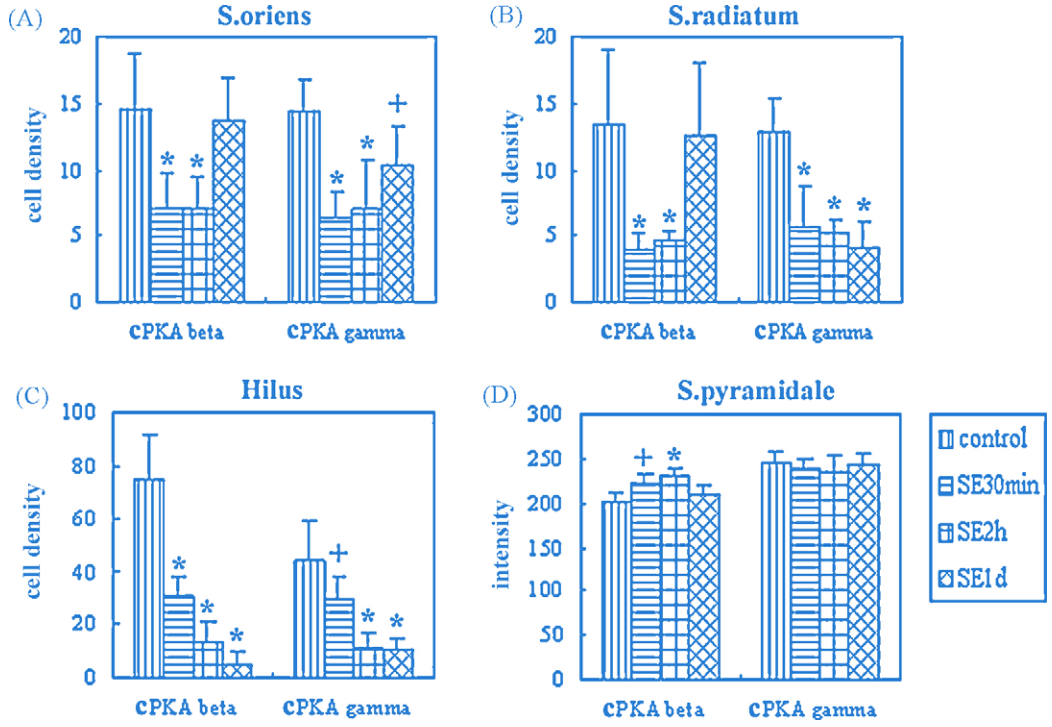

Fig. 4. Histograms showing the progressive changes of cPKAβ or cPKAγ immunopositive neurons/product within 1 day during and after pilocarpine induced status epilepticus. $n = 18$ observations from 6 rats. $^{*}p < 0.01$, $^{+}p < 0.05$ compared with the control. Cell density is indicated as a number per square millimeter; intensity of immunpositive product is indicated as a grey value. Note that the increase of intensity (grey value) indicates the decrease of protein expression, the two are negatively coupled.

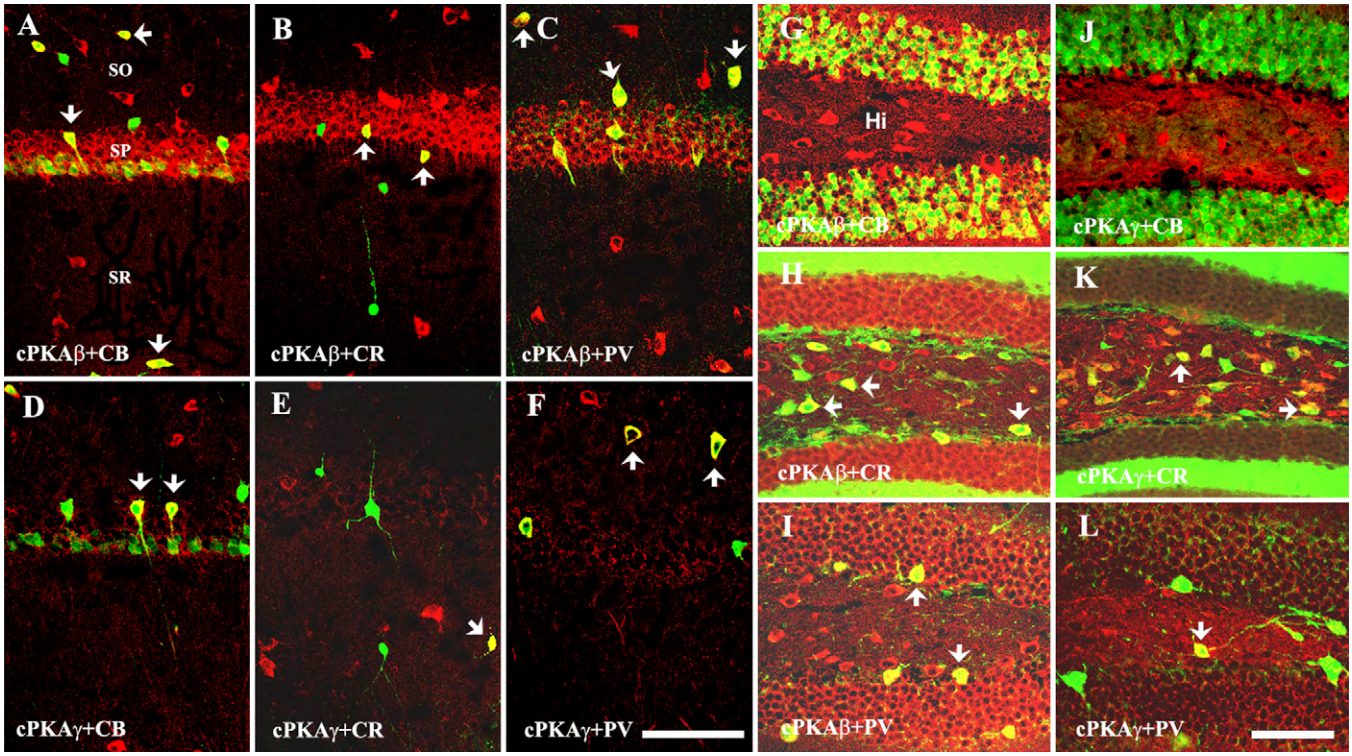

Fig. 5. Double-labeling immunofluoresence microscopy shows co-localization (yellow, arrows) of cPKAβ (A–C, red) or cPKAγ (D–F, red) with CB (A, D, green), CR (B, E, green) or PV (C, F, green) in CA1 area of the hippocampus of the control mouse. In the hilus of the dentate gyrus, co-localization (yellow, arrows) of cPKAβ (G–I, red) or cPKAγ (J–L, red) with CB (G, J, green), CR (H, K, green) or PV (I, L, green) is also indicated. Note that there is not co-localization of cPKA with CB (G–J, green) in hilar neurons. (F) Scale bar = 100 μm, also applies to (A–E); (L) scale bar = 100 μm, also applies to (G–K). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

of CA1 area did not change significantly compared to the control (cPKAβ: Fig. 2A; cPKAγ: Fig. 2E).

3.3.2. Dentate gyrus

3.3.2.1. Stratum granulosum. cPKAβ immunopositive product in the stratum granulosum at 30 min (Fig. 3B), 2 h during (Fig. 3C) and 1 day after (Fig. 3D) pilocarpine induced status epilepticus was similar to the control mice (Fig. 3A). No obvious change of cPKAγ immunopositive product was observed in this layer among different groups of mice (control: Fig. 3E; 30 min: Fig. 3F; 2 h: Fig. 3G; 1 day: Fig. 3H).

3.3.3. Hilus

In the hilus of the dentate gyrus, drastic reduction of both cPKAβ or PKAγ immunopositive neurons occurred at 30 min (cPKAβ: Fig. 3B; cPKAγ: Fig. 3F) and 2 h during (cPKAβ: Fig. 3C; cPKAγ: Fig. 3G) and 1 day after (cPKAβ: Fig. 3D; cPKAγ: Fig. 3H) pilocarpine induced status epilepticus compared to the control mice (cPKAβ: Fig. 3A; cPKAγ: Fig. 3E) ($P < 0.01/0.05$, Fig. 4C).

Table 1
Percentage of calbindin, calretinin or parvalbumin and PKA or PKA double-labeled neurons to the total calbindin, calretinin or parvalbumin immunopositive neurons in CA1 area and in the hilus of the dentate gyrus in the control animals by double immunofluoresence labeling.

Antibodies	CA1 (mean ± SD)	Hilus (mean ± SD)
PKAβ + CB	76.17 ± 6.83	
PKAβ + CR	40.83 ± 6.17	79.5 ± 8.83
PKAβ + PV	95.50 ± 3.17	97.17 ± 2.83
PKAγ + CB	49.50 ± 6.63	
PKAγ + CR	28.83 ± 6.79	79.33 ± 7.45
PKAγ + PV	79.83 ± 5.04	56.33 ± 7.39

3.4. Double-labeling immunofluorescence microscopy

In CA1 area of the hippocampus in the control mice, most of calbindin (CB; Fig. 5A) or parvalbumin (PV; Fig. 5C) immunopositive neurons were cPKAβ (Fig. 5A–C) immunopositive, only 40.8% of CR (CR; Fig. 5B) immunopositive neurons were cPKAβ immunopositive. Most PV (Fig. 5F) immunopositive neurons expressed cPKAγ (Fig. 5D–F), while 49.5% CB (Fig. 5D) immunopositive neurons and 28.8% CR (Fig. 5E) immunopositive neurons were cPKAγ positive (Table 1).

In the dentate gyrus, cPKAβ was co-localized with CB in the stratum granulosum (Fig. 5G). However, there was no co-localization of cPKAγ with CB (Fig. 5J). In the hilus of the dentate gyrus, about 79.5% and 79.3% of CR immunopositive neurons expressed cPKAβ (Fig. 5H) and cPKAγ (Fig. 5K) respectively. Almost all the PV immunopositive neurons were cPKAβ immunopositive in the hilus and the stratum granulosum of the dentate gyrus (Fig. 5I). However, only 56.3% of PV immunopositive neurons were cPKAγ immunopositive (Fig. 5J–L).

4. Discussion

4.1. cPKA in the mouse hippocampus

cPKA has been involved in regulation of $GABA_A$,[14,15] kainate,[16] H2 histamine,[11] NMDA receptor,[7,17] AMPA[18] and type 1 cannabinoid[19] receptors. It also modulates slow Ca^{2+}-activated K^+ current by norepinephrine, serotonin, histamine,[20] corticotropin releasing factor, vasoactive intestinal peptide, calcitonin gene-related peptide,[1] endogenous PGE2,[3] and dopamine[21] in hippocampal neurons.

In situ hybridization has shown that the mRNA expression pattern of cPKAα varied from that of cPKAβ in the hippocampus. mRNA encoding cPKAα was equally distributed between the

dentate gyrus and pyramidal cell layers CAl-3. In contrast, although cPKAβ expression was high in the dentate gyrus, little cPKAβ mRNA was observed in the CAl-3 cell layers.[22] It is inconsistent with our study showing moderately to strongly expressed cPKAβ protein in the stratum pyramidale of CA1-3, suggesting that gene and protein expressions may not be always matched. In subcellular elements of cultured hippocampal neurons, cPKA was co-localized with microtubules in dendrites as well as with the actin filaments in growth cones, which indicated that PKA was anchored to actin and MT cytoskeletons.[23] However, so far, there is still no systemic study of the localization of cPKA proteins in different areas of hippocampus and different types of hippocampal neurons. In the present study, we showed that cPKAβ was expressed by hippocampal principal and hilar cells, interneurons, and mossy fibers, cPKAγ was localized in hippocampal interneurons, hilar cells, mossy fibers and molecular layer of the dentate gyrus. The study therefore provides first neuroanatomical basis for understanding the mechanism of not only functional changes of hippocampal principal cells and interneurons regulated by cPKA,[1,2] but also the selective defect in mossy fiber LTP after ablation of gene targeting on cPKA[24] as cPKAβ is localized in mossy fibers and terminals in the stratum lucidum of CA3 area.

Double-labeling immunohistochemistry revealed that most of PV immunopositive neurons expressed cPKAβ or cPKAγ in CA1 area and in the dentate gyrus, and most of CR immunopositive neurons in the hilus of the dentate gyrus were cPKAβ or cPKAγ immunopositive. It therefore suggests that cPKA may play a very important role in regulation of the activity of PV interneurons (basket cells) which innervate the perisomatic area of principal cells and hilar mossy cells labeled by CR.

4.2. Progressive changes of cPKA in hippocampal neurons during and after pilocarpine induced status epilepticus

Bracey et al.[25] demonstrated an increase in PKA activity in cortical structures, but not in hippocampus at later stages after pilocarpine induced status epilepticus. The present study may therefore provide some clues for better understanding the mechanism of PKA-related neuronal excitability and epileptogenesis during and after status epilepticus.

It has been reported that cPKA inhibits the function of brain $GABA_A$ receptors,[14] and is involved in $GABA_A$ receptor dysfunction in CA1 pyramidal neurons following chronic benzodiazepine treatment.[15] PKA phosphorylation of Kv3.2-containing potassium channels in inhibitory interneurons is also involved in H2 histamine receptor modulated outward currents, which lowered the maximum firing frequency of inhibitory neurons, and in turn negatively modulated high-frequency population oscillations recorded in principal cell layers.[11] In tottering mouse brain, an abnormal elevation of PKA activity contributes to an impairment of $GABA_A$ receptor function which may play a role in induction of the seizures.[5] However, reduced PKA-mediated phosphorylation protects against picrotoxin seizures by increasing the inhibitory potential of $GABA_A$ receptors.[8] In the present study 30 min and 2 h during pilocarpine induced status epilepticus, fewer cPKAβ immunopositive principal cells and cPKAβ or cPKAγ immunopositive interneurons in CA1 area, and fewer cPKAβ or cPKAγ immunopositive hilar neurons in the dentate gyrus were observed, which in fact was not caused by neuronal loss,[26] suggesting a down-regulation of cPKA may reflect a compensatory mechanism to reduce impairment of $GABA_A$ receptor function in the hippocampus. In the hilus of the dentate gyrus, significant reduction of cPKAβ or cPKAγ immunopositive neurons at 1 day after pilocarpine induced status epilepticus may be due to the loss of hilar neurons which has been reported previously.[27,28]

Many of previous studies suggested that cAMP/PKA signaling could modulate excitatory synaptic transmission in the hippocampus. Activating cAMP/PKA signaling pathway enhanced whole-cell current responses to glutamate, and these responses were blocked by a specific peptide inhibitor of PKA.[29] Also, Sp-cAMPS, a phosphodiesterase-resistant activator of PKA, was demonstrated to potentiate glutamatergic receptor currents in cultured hippocampal neurons.[16] Furthermore, single-channel activity monitored in the presence of purified cPKA also showed that these subunits increased opening frequency and mean open time of glutamate receptor channels.[29] Hence, the down-regulation of PKAβ in principal cell of CA1 area at early stage during pilocarpine induced status epilepticus may reduce the presynaptic glutamate release and decreases phosphorylation on serine and threonine residues of $GABA_A$ receptors, which keep GABA receptors consistently below their full inhibitory potential[30] leading to the hyperexcitability of CA1 pyramidal neurons. In the hilus of the dentate gyrus, as most of CR (which is a marker for hilar mossy cells) immunopositive neurons were cPKAβ or cPKAγ immunopositive, down-regulation of cPKAβ or cPKAγ at 30 min and 2 h during pilocarpine induced status epilepticus may reflect a compensatory neuroprotective mechanism to prevent the loss of mossy cells in the dentate gyrus.

In the present study, the quantitative study of protein expression of cPKA has been explored only in dorsal hippocampus instead of the whole hippocampus. Further study may be needed to show if similar changes occur in the ventral hippocampus.

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