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Epileptiform response of CA1 neurones to convulsant stimulation by cyclothiazide, kainic acid and pentylenetetrazol in anaesthetized rats

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ABSTRACT

We have previously reported that cyclothiazide (CTZ) evokes epileptiform activities in hippocampal neurons and induces seizure behavior. Here we further studied *in vivo* the sensitivity of the hippocampal CA1 neurons in response to CTZ in epileptogenesis in comparison with two other classic convulsants of kainic acid (KA) and pentylenetetrazol (PTZ).

CTZ administered intracerebral ventricle (i.c.v.) induced epileptiform activities from an initial of multiple evoked population spikes, progressed to spontaneous spikes and finally to highly synchronized burst activities in hippocampal CA1 neurons. PTZ, when given by subcutaneously, but not by intracerebral ventricle injection, evoked similar progressive epileptiform activities. In contrast, KA given by i.c.v. induced a quick development of epileptiform burst activities and then shortly switched to continuous high frequency firing as acute status epilepticus (ASE). Pharmacologically, alprazolam, a high-potency benzodiazepine ligand, inhibited CTZ and PTZ, but not KA, induced epileptiform burst activities while GYKI 53784, an AMPA receptor antagonist, suppressed CTZ and KA but not PTZ evoked epileptiform activities.

In conclusion, CTZ and PTZ induced epileptiform activities are most likely to share a similar progressive pattern in hippocampus with GABAergic mechanism dominant in epileptogenesis, while CTZ model involves additional glutamate receptor activation. KA induced seizure in hippocampus is different to that of both CTA and PTZ. The results from this study indicate that hippocampal neurons respond to various convulsant stimulation differently which may reflect the complicated causes of the seizure in clinics.

1. Introduction

Using chemically induced models to study epilepsy is now the most widely used way in epilepsy research and screening of novel antiepileptic drugs. However, the surprising fact is that we are still unclear of how exactly these models work. What we know now is that most of these models are founded based on the basal theory which states the fundamental cause of epilepsy is the imbalance between CNS excitatory and inhibitory systems.[1–3] Chemical-induced models, including kainic acid (KA) and pentylenetetrazol (PTZ) models, often use different convulsant to mimic excitatory or inhibitory stimulus to enhance system excitation or suppress proper inhibition of the network which finally induce hyper-excitation towards seizures.

Cyclothiazide (CTZ) seizure animal model is a new chemical convulsant model recently developed in our lab based on its dual action mechanism in driving the central nervous system towards hyper-excitation.[4–6] CTZ is first known to act as an AMPA receptor desensitization blocker, which can prolong glutamate excitatory responses[7–10] as well as increase presynaptic glutamate release.[11–13] Later, it has been demonstrated that CTZ could also directly inhibit $GABA_A$ receptor function, acting as a $GABA_A$ receptor blocker.[14] We recently characterized the convulsant property of CTZ both *in vitro* and *in vivo* to demonstrate that CTZ could induce epileptiform bursts in hippocampal neurons,[4,5] partly due to down regulation of tonic $GABA_A$ receptor function[15] and enhancement of AMPA receptor function,[4] which is also involved in BDNF-TrkB signaling pathway.[5] Recently, we further reported that CTZ could induce seizure behavior in freely moving rats.[6]

PTZ is a noncompetitive $GABA_A$ receptor antagonist and repeated subconvulsive doses of PTZ administration induced a

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chemical-kindling model in both rats and mice,[16,17] and this model has been widely used as a routine test in screening anticonvulsants.[18] In both *in vivo* and *in vitro* preparations, PTZ administration caused hippocampal evoked population spike to form double or multiple peaks[19,20] a sign of the epileptogenesis in hippocampus. It was also shown that PTZ-kindling can induce long-lasting physiological changes within hippocampal CA1 area.[21]

KA is an agonist of kainite subtype of glutamate receptors and was first established as a convulsant to induce epilepsy model by Nadler and colleagues in 1978.[29] It induced repetitive seizures and neuronal damage in hippocampus and amygdala.[22,23] A chronic epilepsy state after latent period following status epilepticus is now widely used in seizure studies. Previous studies also proved that i.c.v. KA injection could induce lesion easily and neuronal cell death in rodent hippocampus.[24,25] Electrophysiological studies showed that KA treatment increased dendritic excitability in hippocampal CA1 and enhanced EPSP/PS coupling.[26–28]

Thus, the current study was designed to compare the epileptogenesis differences among the CTZ, PTZ and KA induced seizure models. By studying the *in vivo* hippocampal CA1 neuronal response to these three different convulsants, we demonstrated that CTZ and PTZ, but not KA, induced epileptiform activities shared similar epileptogenesis process. The pharmacological study also revealed that GABAergic mechanism was dominant for CTZ and PTZ induced seizure activities with additional AMPA receptor involvement for CTZ model.

2. Methods and materials

2.1. Animal preparation

All experiments were performed on urethane anaesthetized (1.2 g kg^{-1}, i.p.) male Sprague–Dawley rats (220–350 g). The level of anaesthesia was assessed by the absence of paw withdrawal reflex, and additional anaesthetic (urethane, 0.2–0.6 g kg^{-1}, i.p.) was administered as necessary. Body temperature was maintained at 37 ± 0.5 °C with a Harvard Homoeothermic Blanket (Harvard Apparatus Ltd., Kent, UK). Animals were housed in a regulated environment (21 ± 1 °C) with a 12 h light–dark cycle, and food and water available *ad libitum*. All experiments were approved by the local committees of The Use of the Laboratory Animals, Fudan University and carried out in accordance with Chinese National Science Foundation animal research regulation. At the end of experiments, animals were euthanized with overdose of urethane.

Animals were prepared as previously described.[4,5,29] Animals had their lateral tail vein cannulated for drug administration and were mounted in a stereotaxic frame. An incision was made in the midline of the head to expose the top part of the skull. For implantation of the i.c.v. cannula, a drill hole was made on the skull above the left side of the lateral ventricle (0.3 mm posterior to bregma, 1.3 mm lateral to the midline). A guide cannula (Plastic One, USA) was then placed 4 mm below the skull surface for drug delivery, and secured by the dental cement.

2.2. Electrophysiological recording and data acquisition

For recording and stimulation, a large burr hole was made in the left side of the incised skull above the hippocampal area, and the dura was pierced and removed. The stereotaxic coordinates were determined from the stereotaxic atlas of the rat brain.[30] A concentric bipolar metal electrode (Harvard Apparatus Ltd., Kent, UK) was placed close to CA3 region (3.8–4.5 mm posterior to bregma, 3.5–4.0 mm lateral to the midline, and 3.0–3.8 mm below the brain surface) in order to stimulate the CA3 pyramidal cell layer and/or Shaffer collateral pathway. For recording in the CA1 pyramidal cell layer, a tungsten electrode (0.5 MΩ, WPI, Stevenage, UK) was placed 3.5–4.2 mm posterior to bregma, 2.0–3.0 mm lateral to the midline. The depth of the recording electrode was approximately 2.0–2.5 mm below the brain surface as determined by the sudden change of the electrical noise and the shape of the evoked field excitatory postsynaptic potential (fEPSP) and population spike (PS). For CA3-CA1 Shaffer collateral stimulation, a constant current generator passed a square-wave pulse (0.2 ms in duration) through the stimulating electrode (test pulse). Test pulses evoked a positive excitatory postsynaptic field potential in the CA1 with a population spike superimposed as a negative deflection on the rising phase. After initial recording and stimulation tests, both electrodes were adjusted to obtain the maximal evoked EPSP and population spike amplitude. The PS amplitude was used as a measure of postsynaptic responses, with a stimulation intensity set at the current required to produce the maximal response, predetermined by input–output curves. During experiments, CA1 pyramidal cell excitability was sampled every 60 s by a test pulse. In-between stimulations, the baseline activity was recorded for evidence of spontaneous activity. The electrophysiological signals were amplified (200 times) and filtered (0.3–3 kHz) using a NeuroLog System (Digitimer Ltd., Hearts, UK) and visualised and stored in a computer through an A–D converter, CED 1401 micro (Cambridge Electronic Design, Cambridge, UK). Once both electrodes were in the right place, the fEPSPs and PS were monitored for at least 30 min (20 min of baseline recording without stimulation and 10 min evoked EPSP/PS recording) until a stable recording was achieved. Following a 30 min recorded baseline of all responses, drugs or vehicles were administered slowly over a period of 10 min by intracerebral ventricle (i.c.v.) injection (CTZ, PTZ and KA) via the pre-implanted guide cannula into the lateral ventricle or by subcutaneous (s.c.) injection (PTZ). Pharmacologically induced epileptiform activity was monitored for at least 3 h after injection by observing changes of evoked potentials transforming from single PS into multi-peaked display and spontaneous epileptiform burst activity in CA1 pyramidal neurons.[27] The anaesthetic level was monitored and maintained throughout the course of experiments, in particular, after convulsant drug administration.

2.3. Analysis of data

Epileptiform activity of CA1 pyramidal cells was analyzed offline using Spike2 (an analysing program for CED 1401, Cambridge, UK) and specific scripts designed for this study with Spike2. Since multiple population spike (PS) peaks typically represent epileptiform evoked responses, the evoked multiple PS peaks, defined as the negative deflection peaks superimposed on the rising phase of the evoked EPSPs, were counted. The spontaneous high amplitude spiking events (>0.5 mV) were defined as those containing 1 or 2 spikes occurring at low frequency (<1 Hz). The latency for spontaneous high amplitude spikes was recorded from the usual 'silent' baseline. The highly synchronized bursting activity was defined, in distinguishing from spontaneous spiking events, as having high frequency multiple high amplitude spikes (>0.5 mV) with an initial interspike interval of less than 0.2 s, a minimum of 5 spikes, and burst duration over 1 s.[5]

Group data were expressed as the mean ± SEM. Across groups of data, statistical significance between means was determined using one-way ANOVA with Tukey HSD post hoc analysis (GraphPad Prism, GraphPad Software Inc.). Comparisons within a group used a paired two-tailed *t* test. Significance level was set at $P < 0.05$.

2.4. Histology, drugs and solutions

The following drugs were freshly made before each experiment: KA (1 μg in 2.5 μl ACSF for i.c.v. injection). Cyclothiazide (5 μmol

in 5 μl DMSO for i.c.v. injection) and PTZ (383 mg ml^{-1} in saline for i.p. injection, 50 μmol in 5 μl ACSF for i.c.v. injection) were purchased from Tocris (Northpoint, Bristol, UK); Pontamine Sky Blue dye (20 mg ml^{-1}; BDH, Poole) was dissolved in 0.5 M sodium acetate; urethane (25%; Sigma–Aldrich Chemical Co., Poole, Dorset) was dissolved in distilled water; alprazolam (10 mg ml^{-1} 1 in normal saline) was supplied by the Huashan Hospital (Fudan University, Shanghai); GYKI 53784 (5 mg ml^{-1} in normal saline for i.v. injection) was a gift from Eli Lilly company.

At the end of experiments, Pontamine Sky Blue (1 μl) was injected through the pre-implanted cannula to verify the cannula position and drug diffusion area within the brain before the brain was removed and fixed in 10% formal saline. Frozen sections (80 μm) were cut. The Pontamine Sky Blue marked sites while the stimulating and recording electrode tracks were visualized and mapped onto standard sections of the brain[30] similarly as reported previously.[5,6] For those successfully recovered recording/stimulating tracks, they were all localized in the right place in CA1 pyramidal cell layer and CA3 area, respectively, and the marked cannula sites were in the lateral ventricle.

3. Results

3.1. CTZ, PTZ and KA induced progressive multiple population spike peaks in anaesthetized rats

In anaesthetized rats, recordings were made from CA1 pyramidal cell layer with stimulating rate at 1/60 s in the area of CA3. In control condition, there was only one single downward population spike (PS) embedded on top of the evoked upward EPSP similar as previous reported[4,5] (Fig. 1A).

CTZ (5 μmol in 5 μl DMSO), microinjected into left-side lateral ventricle, induced time-dependent progressive increases of evoked PS-peak from a single peak to double and then multiple peaks (Fig. 1A). CTZ injection caused the evoked PS to progress to 2nd and 3rd or more (multiple) peaks in all 13 rats studied and the latency for 2nd and multiple peaks to appear was 17.3 ± 1.8 min and 34.9 ± 3.8 min, respectively (Fig. 3A).

In contrast, PTZ (50 μmol in 5 μl ACSF), microinjected into left-side lateral ventricle, neither evoked PS peak nor the spontaneous activity (see below) change within the 3-h recording period. This result indicates that PTZ is lack of epileptogenic effect when given i.c.v. Since many reports have shown systemic administration of PTZ induced seizures,[31,32] next, we studied whether PTZ given by subcutaneous injection could induce epileptiform activities. Indeed, PTZ (383 mg kg^{-1}, s.c.), similar as CTZ, induced time-dependent progressive increases of evoked PS-peak from a single peak to double and then multiple peaks (Fig. 1B). PTZ injection caused the evoked PS to progress to 2nd and 3rd or more (multiple) peaks in all 14 rats studied and the latency for 2nd and multiple peaks to appear was 9.7 ± 0.8 min and 27.3 ± 4.1 min, respectively (Fig. 3A).

As CTZ and PTZ shared a similar epileptiform progressive character in evoked action potentials, we then tested another classic convulsant compound kainic acid. KA (1 μg in 2.5 μl ACSF), in contrast to CTZ and PTZ, only induced 2 in 10 rats tested to progress to 2nd and multiple peaks. In the other 8 rats tested, the burst activity rapidly occurred after KA injection (see below) and then the EPSP and PS were not able to be evoked after bursts (Fig. 1C).

As control for intra-ventricle vehicle injection, neither DMSO (for CTZ, 5 μl, $n = 6$) nor ACSF (for KA, 2.5 μl, $n = 3$) induced any changes on evoked population spikes.

3.2. CTZ, PTZ and KA induced spontaneous epileptiform activity in anaesthetized rats

The baseline activity, before the convulsant drug administration, of the recordings from CA1 pyramidal cell layer was normally "silent", with no detectable activities such as spikes above the baseline, in anaesthetized rats (Fig. 2), similar as previous reported.[4,5]

CTZ (5 μmol in 5 μl DMSO), microinjected into left-side lateral ventricle, after a delay, induced high amplitude, isolated spikes, and then gradually progressed into highly synchronized epileptiform bursts (Fig. 2A). The latencies for the first spontaneous spike and the first burst to occur after CTZ administration were 53.9 ± 4.3 min ($n = 13$) and 111.7 ± 14.6 min ($n = 13$), respectively (Fig. 3B). Usually, the burst activities were relatively stable and lasted beyond the experiment ending (>3 h after drug administration). Once the bursting activity occurred, it was usually impossible to evoke EPSPs and PS.

PTZ (50 μmol in 5 μl ACSF, $n = 3$) did not make any change in the spontaneous activities within 3 h recording period (data not

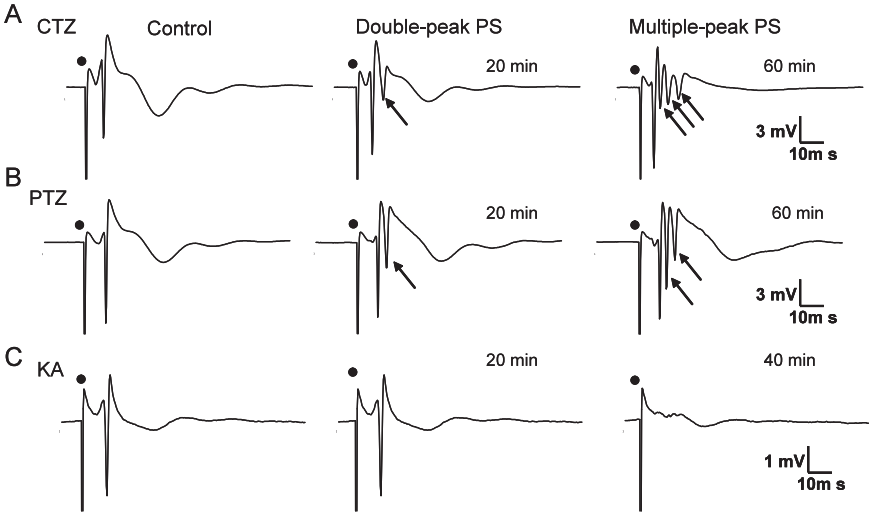

Fig. 1. Progressive change of the epileptiform hippocampal CA1 evoked potentials induced by CTZ, PTZ and KA. Original traces showing the number of the population spike (PS) peaks embedded in the evoked EPSPs in CA1 pyramidal cell layer were transformed, time dependently, from single peak to multiple peaks (arrow indicated) by administration of (A) CTZ (5 μmol, i.c.v.) and (B) PTZ (383 mg kg^{-1}, s.c.), but not by (C) KA (1 μg, i.c.v.) (●, stimulus artifact). (*Note*: KA not only inducing multiple PS peaks, but also blocked evoked EPSP-PS after status epilepticus occurring.)

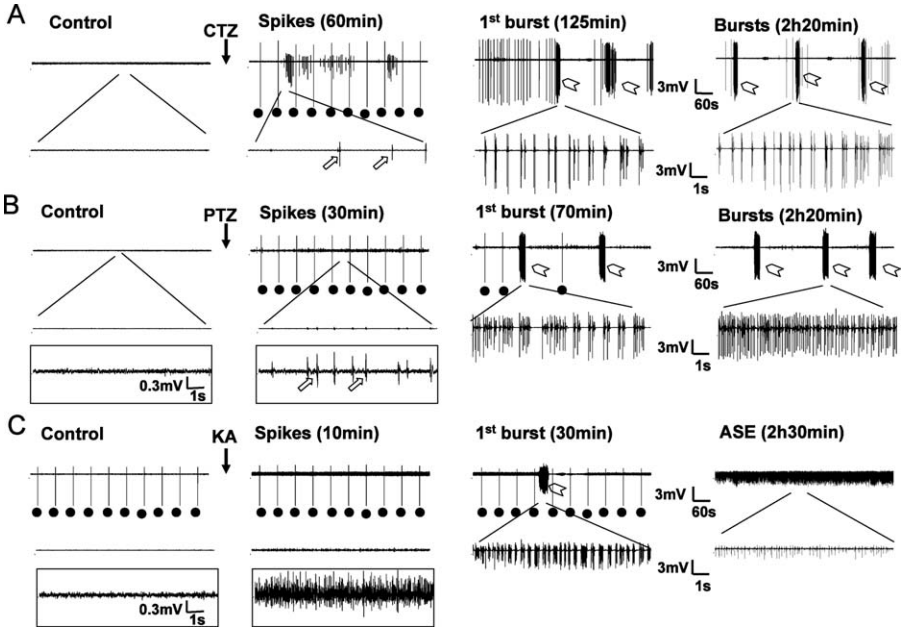

Fig. 2. Progressive pattern of the epileptiform hippocampal CA1 activities induced by CTZ, PTZ and KA. Field potential recordings of the spontaneous hippocampal CA1 neuronal activities before and after CTZ, PTZ and KA stimulation. (A and B) Original traces showing (from left to right) the control baseline activity, spontaneous spikes (open arrows) and first synchronized bursts (arrow head) occurring and progressed to consistent bursts before and after (A) CTZ (5 μmol, i.c.v.) and (B) PTZ (383 mg kg^{-1}, s.c.) injection. (C) Original traces showing (from left to right) the control baseline activity, high amplitude spontaneous activities, synchronized bursts and progressed to ASE before and after KA (1 μg, i.c.v.) injection. Note, the traces under the original traces are expanded to show the detail of the activities and the traces in the box are enlarged to view the activities (●, stimulus artifact).

shown). In contrast, PTZ (383 mg kg^{-1}) given by s.c. induced similar change as CTZ injected by i.c.v. with induced high amplitude and isolated spikes, and then gradually progressed into highly synchronized epileptiform bursts (Fig. 2B). The latency for the occurrence of the first spontaneous spike and the first burst after PTZ administration were 30.9 ± 2.2 min ($n = 14$) and 48.2 ± 5.0 min ($n = 14$), respectively (Fig. 3B). Usually, the burst activities were relatively stable and lasted beyond the experiment ending (>3 h after drug administration). Once the bursting activity occurred, it was usually unable to evoke EPSPs and PS similar as that of CTZ.

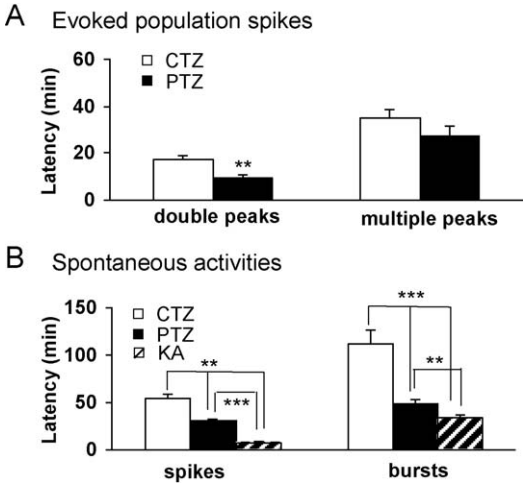

Fig. 3. Group data of CTZ, PTZ and KA induced epileptiform activity latency. Bar histograms showing the group data of the latency of epileptiform activities induced by CTZ (5 μmol, i.c.v.), PTZ (383 mg/kg, s.c.) and KA (1 μg, i.c.v.). (A) Comparison of the latency for evoked population spikes progressed to double peaks and multiple peaks (≥3 peaks) between CTZ and PTZ. (B) Comparison of the latency for spontaneous spikes and synchronized bursts among CTZ, PTZ and KA. $^{**}P < 0.01$, $^{***}P < 0.001$

In contrast, KA (1 μg in 2.5 μl ACSF, i.c.v.) evoked quick change of the baseline spontaneous activities with the appearance of the high amplitude spikes during KA injection period (see Section 2) and shortly transformed to highly synchronized burst discharges, which was then followed by continuously firing spikes with high amplitude and frequency, defined as acute status epilepticus (ASE) (Fig. 2C). The latency for the first spontaneous spike and the first burst to occur after KA injection was 8.9 ± 1.0 min ($n = 10$) and 33.6 ± 3.2 min ($n = 10$), respectively (Fig. 3B). In contrast to the CTZ and PTZ induced stable burst discharges, KA induced burst discharges lasted relatively much shorter, with an average bursted period of 33.9 ± 5.7 min and contained 7 ± 1 bursts, ranging from 3 to 13 bursts in 10 rats studied. The burst activities were then transformed to ASE discharges, which usually lasted for more than 5 min and, if stopped, quickly started again with a very short gap. The latency for the appearance of ASE discharges was 70.6 ± 7.5 min and lasted beyond the experimental ending (>3 h after KA injection).

As control for intra-ventricle vehicle injection, neither DMSO (for CTZ, $n = 6$) nor ACSF (for KA, $n = 3$) induced any changes on baseline activities.

3.3. Effect of alprazolam on epileptiform activities induced by CTZ, PTZ and KA

To test the pharmacological differences among these three chemical induced epileptogenic animal models, we first studied the effect of alprazolam, a highly potent short-acting drug of the benzodiazepine class, which was used clinically as an anticonvulsant,[33,34] on the burst/ASE status induced by CTZ, PTZ and KA in anaesthetized rats.

Alprazolam was administrated (0.4 mg kg^{-1}, i.p.) three hours after either CTZ (5 μmol, i.c.v.) or PTZ (383 mg kg^{-1}, s.c.) treatment, if there was stable bursting activities existing for more than 30 min, or after KA (1 μg, i.c.v.) treatment, if the ASE was stably existed. Alprazolam significantly suppressed both the CTZ ($n = 5$) and the PTZ ($n = 8$) induced epileptiform burst frequency

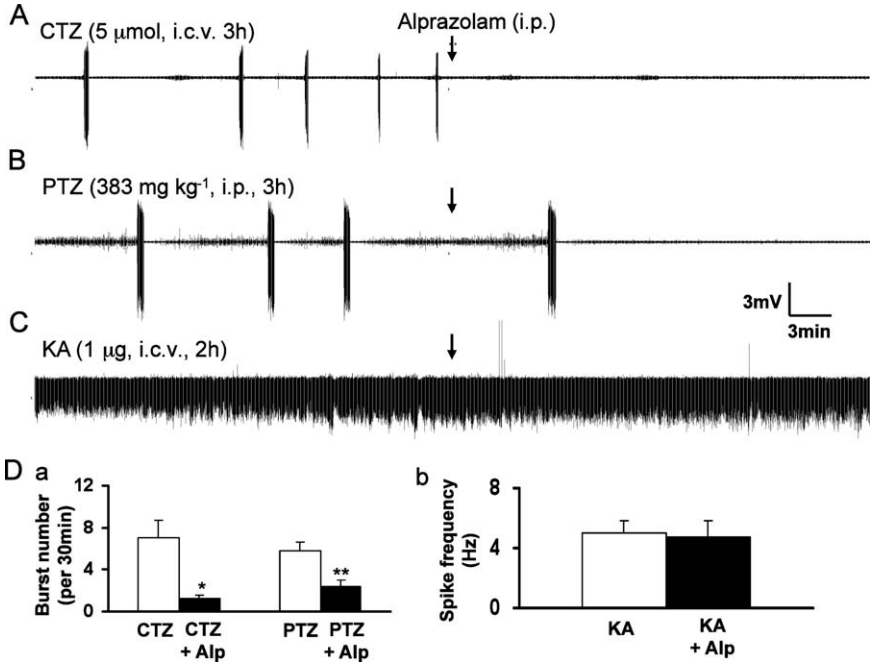

Fig. 4. Effect of alprazolam on CTZ, PTZ, KA induced epileptiform activities in hippocampal CA1 neurons. (A–C) Original traces showing CTZ and PTZ induced epileptiform burst and KA induced ASE activities before and after alprazolam (0.4 mg kg^{-1}, i.p.) injection. Alprazolam inhibited CTZ (A) and PTZ (B) induced bursts but not KA-induced ASE spikes (C). (D) Bar histogram showing the group data of alprazolam inhibition on CTZ ($n = 5$) and PTZ ($n = 8$) induced burst activities (a) but no effect on KA ($n = 9$) induced ASE firing (b). $^{*}P < 0.05$, $^{**}P < 0.01$.

(Fig. 4A and B). The epileptiform bursts were reduced from 7.0 ± 1.6 to 1.2 ± 0.4 (bursts/30 min, $P < 0.05$) in CTZ group and 5.8 ± 0.9 to 2.4 ± 0.6 (bursts/30 min, $P < 0.01$) in PTZ group, before and after alprazolam treatment, respectively (Fig. 4D). In contrast, alprazolam had no effect on KA induced continuous firing spikes (Fig. 4C). The firing frequency before and after alprazolam injection was 5.0 ± 0.8 and 4.8 ± 1.1 Hz ($n = 9$, $P > 0.5$), respectively (Fig. 4D).

3.4. Effect of GYKI 53784 on epileptiform burst activity induced by CTZ, PTZ and KA

Results of alprazolam study showed an effective action on suppressing CTZ and PTZ, but not KA, induced epileptiform bursts by modulation of the GABA system. As CTZ also involves the action on AMPA receptors, we then applied GYKI 53784, a non-competitive AMPA receptor antagonist, to test responses of CTZ, PTZ and KA induced epileptiform activities.

GYKI 53784 was administrated (5 mg kg^{-1}, i.v.) three hours after either CTZ (5 μmol, i.c.v.) or PTZ (383 mg kg^{-1}, s.c.) treatment, if there were stable bursting activities existing for more than 30 min, or 30 min after KA induced stable ASE firing. GYKI 53784 significantly suppressed CTZ but not PTZ induced epileptiform burst frequency (Fig. 5A and B). The epileptiform bursts induced by CTZ was reduced from 6.4 ± 2.1 to 1.2 ± 1.0 (bursts/30 min, $n = 5$, $P < 0.01$) before and after GYKI 53784 injection, respectively (Fig. 5Da). Interestingly, in all 5 rats studied, GYKI 53784 only inhibited the epileptiform bursts to transform the burst activities backwards to the isolated spike firings (Fig. 5A). In contrast, the burst activities induced by PTZ were not affected by GYKI with the burst frequency at 8.6 ± 2.3 and 7.6 ± 1.9 (bursts/30 min, $n = 7$, $P > 0.5$), before and after GYKI 53784 injection, respectively (Fig. 5C). In addition, GYKI 53784 (5 mg kg^{-1}, i.v.) also significantly inhibited KA induced ASE firing in all 4 rats tested (Fig. 5C). The established ASE spike firing frequency was reduced from an average of 7.3 ± 1.6 Hz to 1.4 ± 1.2 Hz ($n = 4$, $P < 0.01$), before and after GYKI administration, respectively (Fig. 5Db).

4. Discussion

This study demonstrated that CTZ, PTZ and KA are all capable of induce epileptiform activities in hippocampal CA1 pyramidal neurons in anaesthetized rats. However, the neuronal seizure activities induced by these three convulsants showed different progressive patterns, with CTZ and PTZ shared a similar time-dependent development way from initial increases of evoked population spike peaks to the appearance of the spontaneous spike then progress to the highly synchronized bursts. In contrast, KA induced epileptiform activity has a fast development but short lasting of both enhanced baseline activities and the burst activates followed by the long lasting continuous high frequency spike firing, classified as ASE discharges. Pharmacologically, we found that enhanced GABAergic activation inhibited CTZ and PTZ induced epileptiform bursting activities but with no effect on KA evoked ASE discharges. In addition, antagonizing the AMPA receptors not only inhibited KA induced epileptiform activities, but also attenuated CTZ, but not PTZ, induced burst activities, changing the highly synchronized burst firings to spike firing status.

The current study was designed to compare the hippocampal CA1 neuronal response in three seizure animal models, among them the CTZ model was a newly developed model in our lab.[5,6] Since CTZ is a non-brain penetrating compound demonstrated in our previous studies,[4,5] the initial experimental regime was to administer all these three convulsants by i.c.v. for direct comparison. Indeed, both CTZ and KA administered by i.c.v. induced epileptiform activities in the CA1 pyramidal neurons in our anaesthetized rat models. However, to our surprise, when PTZ was given by i.c.v. at the dose upto 50 μmol induced neither evoked PS change nor spontaneous activity appearance, but it did successfully induce epileptiform activities while given systemically (s.c.) as previously well documented.[19,20,35] The lack of effect of PTZ when given centrally was unlikely due to the dose of PTZ being low in our current study. First, the concentration of PTZ given by i.c.v. was already 10 times higher than CTZ (5 μmol), which did

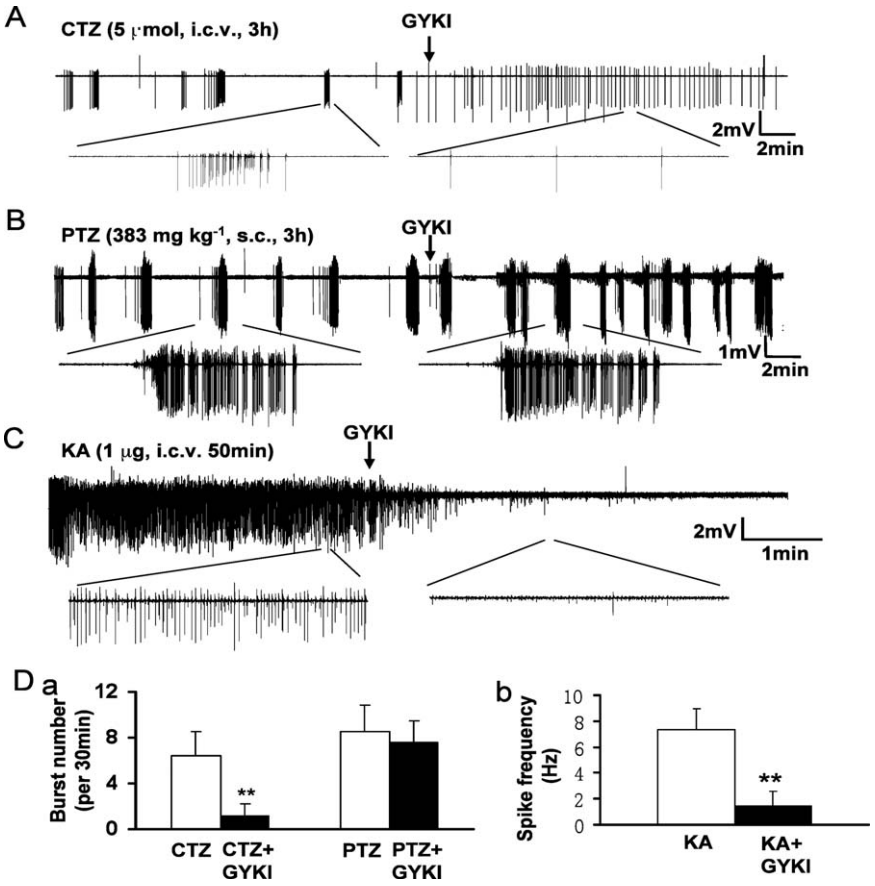

Fig. 5. Effect of GYKI on CTZ, PTZ and KA induced epileptiform activities in hippocampal CA1 neurons. (A–C) Original traces showing CTZ and PTZ induced epileptiform burst and KA induced ASE activities before and after GYKI (5 mg kg^{-1}, i.v.) injection. GYKI inhibited CTZ (A) and KA (C) but not PTZ (B) induced burst activities. (D) Bar histograms showing the group data of GYKI inhibition on CTZ ($n = 5$) but not on PTZ ($n = 7$) induced bursting frequency (a) and inhibition on KA ($n = 4$) induced ASE firing (b). $^{**}P < 0.01$.

successfully induce epileptiform activities, used in the same preparation in our current study. Secondly, the PTZ dose we used (upto 50 μmol) proximally reached to a local concentration of ~25 mM, if the total rat brain extracellular fluid volume was estimated at 2 mL, which was much higher than that commonly used to induce epileptiform activities in *in vitro* concentration controlled studies (3–10 mM of PTZ).[36–38] Thus, we hypothesize that PTZ may work as a convulsant by its metabolites instead while central injection of PTZ failed to produce such metabolites. However, this hypothesis seems contradictory to a previous pharmacokinetic study showing that PTZ, when the cerebrospinal/brain concentration reached proximally ~46 and ~109 mg L^{-1} (~0.23 mM and ~0.55 mM, respectively), was capable of inducing either the onset of the first myoclonic jerk or the maximal seizure, respectively, in rats.[39] The authors claimed that the seizure was induced by PTZ rather than the metabolites. However, it is notable that the PTZ concentration in cerebrospinal fluid induced maximal seizure (~109 mg L^{-1}) was almost 50 times lower than that of in our current study by i.c.v. administration (~25 mM). In addition, in this pharmacokinetic study, since PTZ was given by systemic infusion, it could not distinguish whether the metabolites of the PTZ or PTZ itself contributed to the convulsant action of PTZ. Another argument was that the preparation used was different for conscious rats in Raman and Levy's[39] study and anaesthetized rats in current study. However, in our current anaesthetized animal preparation, systemic administration of PTZ by s.c. at the dose of 110 mg kg^{-1} did indeed successfully induce epileptiform activities such as multiple evoked PS peaks (data not shown) which was not much different to those reported in conscious rodents.[39,40] Thus, we are confident to believe that the dose of PTZ injected by i.c.v. route in current study was high enough, if it acts as a convulsant, to induce epileptiform activities. However, the hypothesis, that the convulsant action of PTZ might be mediated by its metabolites, needs to be further investigated.

Similar as previously demonstrated,[4,5] CTZ given centrally (i.c.v.) induced progressive epileptogenesis: from initial evoked population spike peak transforming from single peak in normal condition towards multiple peaks,[27] to the appearance of the spontaneous high amplitude spikes, and then finally forming the highly synchronized bursting activities. Interestingly, when PTZ was given systemically, it shared the similar epileptogenic progressive pattern. PTZ induced evoked population spike peak transforming from single peak in normal condition towards multiple peaks, and then the spontaneous activities, which was seen in current study is similar to those previously well documented.[27,28] In addition, the burst activities induced by both CTZ and PTZ were relatively stable after their occurrence, at least beyond our experiment regime (>3 h) similar as previous reported for CTZ model,[7] and in some cases, burst activities were still detectable 5–6 h after initial convulsant administration (data not shown). In contrast, while KA was given by i.c.v., it showed totally different epileptiform activity generation pattern with a fast development but short lasting of the burst activity period (~34 min in duration) followed by the long lasting continuous high frequency spike firing. Due to its rapid onset of the induced burst activities after KA injection, both the EPSP and PS were not able to be evoked after bursts similar as previous reported after spontaneous seizure.[41] Thus the evoked potential change, which was normally seen in *in vitro* brain slice study, was also not detectable in our current study. Thus, we hypothesize that the

differences of epileptogenesis among these three convulsants induced epileptiform activities of CA1 hippocampal neurons are likely due to the different pharmacological mechanisms behind these three convulsants.

It is well known that the imbalance between excitation and inhibition is important in the epileptogenic progress.[1–3] Thus, chemical convulsants showed different convulsive effects which might be due to various pharmacological mechanisms. Among them PTZ and KA models are the most widely used classic chemical-convulsant models for epilepsy study and screening of novel anti-epileptic drugs.[25,29,30] PTZ produces its convulsive effect by inhibiting the activity of GABA at $GABA_A$ receptors.[35] KA is a specific agonist at the ionotropic glutamate receptor kainate subtype which mimics the effect of glutamate. Differently from these two classic convulsants, CTZ works through a combination of potentiation of glutamate receptor AMPA subtype function and inhibition of $GABA_A$ receptor response.[7–10,14] This has also been confirmed by our pharmacological results demonstrated in current study. Our results showed that enhancement of the GABA function by modulating the benzodiazepine site of the $GABA_A$ receptors in the CA1 area using alprazolam abolished CTZ and PTZ, but not KA, induced epileptiform bursting activities. In contrast, antagonization of AMPA receptors by GYKI only inhibited CTZ and KA, but not PTZ, evoked epileptiform activities. These data have further supported the widely accepted theory that the excitation/inhibition imbalance is the most important fact in epileptogenesis. Thus the different action site of KA (glutamate receptors) vs CTZ (GABA and AMPA receptors) vs PTZ (GABA receptors) may represent the different seizure induction and development properties of these three convulsants.

Most of the previous studies on chemical convulsants stimulation induced neuronal responses were based on the investigation of the evoked population spike peak transformation.[5,19,20,27] Few reports demonstrated that convulsant stimulation could evoke epileptiform bursting activities either *in vitro* or *in vivo*.[42,43] It is notable in current study that both PTZ and CTZ induced progressive epileptogenesis towards the highly synchronized bursting activity of CA1 pyramidal neurons, but KA stimulation failed to produce highly synchronized bursting activities, instead evoked long lasting high frequency spikes. In addition, our pharmacological experiments also revealed that application of benzodiazepine ligand alprazolam eventually abolished the epileptiform bursts induced by both CTZ and PTZ. These results suggest that down regulation of GABAergic function[15] may be the dominant fact for generating synchronized epileptiform bursting activities by CTZ and PTZ, which was consistent with the notion that GABA activity sets the tune for preventing epileptiform activities[44] and GABA function is essential for neuronal synchronization.[45] However, this hypothesis needs to be further studied, particularly for the CTZ model.

In summary, our current study is the first to directly compare the hippocampal CA1 neuronal responses to CTZ, a newly developed convulsant of generating temporal lobe epilepsy animal model[6] with two classic convulsants, PTZ and KA. The results suggest that the onset and the progress pattern of the epileptiform activities in hippocampal neurons in various seizure animal models are different which is likely attributed to the underline pharmacological mechanisms. This may represent the complexity of the seizure onset and development observed clinically.[46–49] CTZ, a newly discovered convulsant in our lab,[5,6] with its dual action mechanism on both glutamatergic and GABAergic systems, may represent the true nature of one of the fundamental seizure mechanism of imbalance of the glutamate excitation and GABA inhibition in the central nervous system. Thus, CTZ model may provide us a much better seizure animal model to represent the complexity of the clinical seizure for epilepsy study and anticonvulsant drug screening.

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