Ginkgo Biloba Extracts Inhibit Ischemic LTP Through Attenuating EPSCs In Rat Hippocampus

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Abstract

Ginkgo biloba extract 761 (Egb761), a standardized extract from the Ginkgo biloba leaf, is purported to inhibit NMDA receptor-mediated neuronal excitotoxicity and protect neurons form ischemic injury. However, the specific signal pathway involved in the effects of Egb761 on synaptic plasticity is still in dispute. In this article, effects of Egb761 and its monomer component Ginkgolide A (GA), Ginkgolide B (GB) and Ginkgolide C (GC) on rat hippocampal synaptic plasticity were studied. The evoked Excitatory postsynaptic currents (EPSCs) and miniature EPSCs were recorded on hippocampal slices from SD rats (14–21 days of age) by whole-cell patch-clamp recording and long-term potentiation (LTP) was induced by theta-burst stimulation. Acutely applied Egb761 inhibited the LTP, but bilaterally affect the EPSCs, that is EPSC increase at lower concentration of Egb761, then EPSC decrease at higher concentration of Egb761. Egb761 monomer component GA, GB and GC could also inhibit the TBS-induced LTP and EPSC amplitude but not paired-pulse ratio (PPR). Simultaneously, Egb761 and its monomer components inhibited the post-ischemic LTP (i-LTP) by inhibiting the EPSCs and the AMPA receptor subunit GluA1 expression on postsynaptic membrane. The results indicated that high concentration of Egb761 might inhibit LTP and i-LTP through inhibition effects of GA, GB and GC on AMPA receptors.

Introduction

Long-term potentiation (LTP), a mechanism by which information arriving at the neuronal level as a sequence of subthreshold impulses is finally engraved into the receiving cell by an excitatory potential, probably represents the most relevant mechanism for learning and memory, drug addiction, and other physiological and pathological neuronal activities at the cellular level (Lee et al. 2021). Typical signal molecules responsible for the formation of LTP is the glutamate receptor subunit GluA1 (Diering and Huganir 2018), which is phosphorylated and aggregated at post-synaptic membrane and leads to an increase of excitatory post synaptic currents (EPSCs) after a serials of high frequency stimulation (Nicoll and Malenka 1999).

A pathological form of plasticity, named post-ischemic long-term potentiation (i-LTP), was observed in glutamate receptor-mediated neurotransmission after stroke and thought to exert a detrimental effect via facilitation of excitotoxic damage (Calabresi et al. 2003; Costa et al. 2011; Zheng et al. 2017). This long-term enhancement in glutamate receptor-mediated excitatory responses was mainly attributed to excessively released synaptic glutamate and the followed increase of intracellular Ca^{2+} through N-methyl-D-aspartate (NMDA) type glutamate receptors (Wu et al. 2017; Zhou et al. 2015). Given that overexcitation of neurons caused by stroke disturbed the balance between excitation and inhibition, restoring this balance via intervention with additional inhibition seems to be a potential and practical strategy (Ding et al. 2011; Sandu et al. 2017; Wu et al. 2017; Zhou et al. 2015).

Ginkgo biloba, a traditional Chinese medicine with a long history, has various pharmacological activities such as promoting blood circulation and removing blood stasis, dredging collaterals and relieving pain, relieving asthma, removing turbidity and reducing lipid (Chinese Pharmacopoeia). Ginkgo biloba leaf

extract761 (Egb761), an effective extract of Ginkgo biloba leaves from extraction solvent acetone 60% (w/w), is widely used as a dietary supplement due to its beneficial effects on conditions ranging from stroke to mental dysfunction (Ba and Min 2015). Egb761 mainly contains the following active compounds: 24% flavonoids, 13% carboxylic acids, 7% proanthocyanidins, 6% terpenoid lactones, and trace amounts of gallic acid, etc. Among which terpenoid lactones include ginkgolides A (GA), ginkgolides B (GB) and ginkgolides C (GC) (Feng et al. 2019).

As a mixture of many active ingredients, Egb761 has a variety of pharmacological activities. It is currently believed to be antioxidant (Di Meo et al. 2020; Ran et al. 2014), anticancer (Chang et al. 2018), anti-inflammation (Chen et al. 2013; Kaur et al. 2018; Kotakadi et al. 2008), anti-platelet aggregation (Hong et al. 2013), promoting angiogenesis (Pan et al. 2018; Zhu et al 2015), lowering blood lipids (Wang et al. 2019) and so on.

Several studies indicates that effects of Egb761 psychiatry diseases are apparent (Dai et al. 2018; El Tabaa et al. 2017; Kandiah et al. 2019; Singh et al. 2017). It could improve anxiety and depression (Banin et al. 2021), inhibit formation of amyloid-β (Aβ) fibrils (Xie et al. 2014), improve the antioxidant capacity of mitochondria, and improve the occurrence of neurodegenerative diseases caused by mitochondrial damage (Tu et al. 2020). In addition, Egb761 significantly attenuated mitochondria-initiated apoptosis and decreased the activity of caspase3, a key enzyme in the apoptosis cell-signaling cascade [14]. Monomer component of Egb761, GB, could improve the activity of ATPase, which is an important guarantee of energy stability (Goyal et al. 2017).

In study of learning and memory, Egb761 could improve the impaired cognition in Alzheimer's patients or in people experiencing age-related decline in memory (Tan et al. 2015). LTP after impairment by aging or dementia was also improved by Egb761 (Lu et al. 2014; Yeh et al. 2015). However, the molecular mechanism of the effects of Egb761 on the synaptic plasticity, especially the LTP after ischemia is still in dispute.

In this study, we aimed to clarify the effects of Egb761 and its monomer component GA, GB and GC on LTP under physiological conditions and post-ischemic state (i-LTP), and further to study whether the GluA1 is involved in the effects of Egb761 and its monomer components.

Materials And Methods

Animals

Animal maintenance and use were in accordance with guidelines provided by the Experimental Animal Ethics Committee of the Xuzhou Medical University.

Hippocampal Slice Preparation

Sprague Dawley rats, 14 to 21 days after birth, were anesthetized with ethyl ether and sacrificed by decapitation. The entire brain was removed and coronal brain slices (350μm thickness) were cut with a

vibrating blade microtome in ice-cold artificial cerebrospinal fluid (ACSF) containing: (in mmol/L) 119 NaCl, 2.5 KCl, 1.3 $MgCl_2$, 2.5 $CaCl_2$, 1.25 KH_2PO_4, 1.3 $NaHCO_3$, and 20 glucose. The osmotic pressure was 300–320 mOsm and pH was adjusted to 7.4. ACSF was bubbled continuously with Carbogen (95%O_2/5%CO_2). Fresh slices were incubated in a chamber with carbogenated ACSF and recovered at 37°C for at least 1.5 hours before being transferred to a recording chamber.

Oxygen-Glucose-Deprivation-Induced Ischemia Model

Anoxia/hypoglycemia was induced by replacing 95% O_2/5% CO_2 with 95% N_2/5% CO_2 and switching to an ACSF containing 20 mmol/L sucrose instead of glucose for 6 to 10 minutes. Egb761 and its monomer components were administered 30 min before OGD respectively.

Electrophysiological Recording

Conventional whole-cell recordings were made with patch pipettes containing (in mmol/L): 132.5 Cs-gluconate, 17.5 CsCl, 2 MgCl2, 0.5 EGTA, 10 HEPES, 4 adenosine triphosphate (ATP), and 5 QX-314, with the pH adjusted to 7.2 by CsOH and the osmotic pressure was 280–300 mOsm. Synaptic responses were evoked at 0.05 Hz. EPSCs were recorded at -65mV holding potential in ACSF perfusion media containing bicuculline methiodide (10 μmol/L) to block GABA receptor-mediated inhibitory synaptic currents. CA1 neurons were viewed under upright microscopy (ECLIPSE E600-FN, Nomasky, Nikon Corporation) and recorded with an Axopatch-700B amplifier (Molecular Devices). Current data were collected with pCLAMP10.3. Changes in AMPA receptor-mediated EPSC (AMPA EPSC) amplitude were examined during the last 10 minutes of recording. Data were low-pass filtered at 2 kHz and acquired at 5 to 10 kHz. In studies of LTP and i-LTP, recording from each neuron lasted from 60 to 80 minutes. The series resistance in these processes varied between 4 to 8 MΩ. The series resistance was always monitored during recording for fear of resealing the ruptured membrane, which will cause changes in both the kinetics and amplitude of the EPSCs. Cells in which the series resistance or capacitance deviated by more than 20% from initial values were excluded from analysis. Cells with series resistance higher than 20MΩ at any time during the recording were excluded from analysis too.

Separation of TIF fraction

Hippocampal slices were prepared as described above. After recovery, slices were stored in liquid nitrogen immediately or homogenized directly in ice-cold 0.32 mol/L sucrose homogenization buffer (pH7.4) containing (in mmol/L) 10 HEPES, 1 $NaHCO_3$, 1 $MgCl_2$, 20 β-phosphogrycerol, 20 sodium pyrophosphate, 0.2 dithiothreitol, 1 EGTA, 1 EDTA, 50 NaF, 1 Na_3VO_4, and 1 p-nitrophenyl phosphate,, in the presence of protease inhibitors: 1 mmol/L phenyl-methylsulfonyl fluoride (PMSF), 5 μg/ml pepstatin A, 5 μg/ml aprotinin, 5 μg/ml leupeptin, and 16 μg/ml benzamidine. The homogenization was centrifuged at 1,000 × g for 10 min, then 200 μl of the supernatant was kept as total protein fractions (Total) and the left supernatant was centrifuged at 3,000 × g for 15 min to obtain a fraction of mitochondria and synaptosomes. The pellet was resuspended in 8 ml hypotonic buffer with the presence of proteases inhibitors and centrifuged at 100,000 × g for 1 hr. The resulting pellet was resuspended in 8 ml buffer containing 75 mmol/L KCl and 1% Triton X-100 and centrifuged at 100,000 × g for 1 hr. The final pellet

was homogenized by 3 times in 20 mmol/L HEPES buffer containing protease inhibitors and phosphatase inhibitors. This fraction is regarded as Triton-insoluble fraction (TIF). Sample was stored at -80°C or immunoprecipitated and immunoblotted immediately (Ren et al. 2013; Yan et al. 2011).

Immunoblotting

Total or TIF samples prepared as mentioned above were separated by SDS-PAGE using 7.5% gradient gels and transferred onto nitrocellulose filter (NC) membranes. The membranes were firstly blocked by 3% bovine serum albumin fraction V, and blotted with anti-phosphorylated GluA1 antibody or anti-actin antibody. Bands were developed by SuperSignal ECL assay kits (ThermoFisher, Rockford, IL) then stripped and blotted with anti-GluA1 antibody.

Statistical Analysis

All data acquired by patch-clamp recording were expressed as mean ± SEM, The data of mEPSCs were analyzed with Mini-analysis (Synaptosft, Decatur, GA). The frequency and amplitude of mEPSCs were calculated for at least 5 min of recordings. The paired-pulse ratio (PPR) was calculated by dividing the amplitude of the second EPSCs by that of the first EPSCs. Data sets were compared with either Student's t-test or ANOVA followed by Tukey's post hoc analysis. Results were considered to be significant at $P < 0.05$.

Results

Effects of Egb761 on TBS-induced LTP on rat hippocampal CA1 neurous

Evoked EPSCs were recorded by whole-cell patch clamp recording in existence of 10μM Bicuculline during the whole recording process. Theta-burst stimulation (TBS) was used to induce LTP in hippocampal slice CA1 neurons through the shaffer collateral pathway. The EPSCs amplitude recorded 50–60 min after TBS in each group were observed to show the changes of LTP. The results showed that TBS-induced CA1 neuron EPSC amplitude increased after the TBS. High concentration of Egb761 administered 30 min prior to TBS could inhibit the formation and the maintenance of LTP (Fig. 1).

Effects of Egb761 on evoked EPSCs, mEPSCs and PPR on rat hippocampal CA1 neurons

EPSCs were recorded for more than 10min to get a stable baseline, and then slices were incubated by the ACSF containing Egb761 (50, 100 and 250 μg/ml, separately). The amplitude of EPSCs got after 20–25 min perfusion of Egb761 was compared with that of the baseline. Results shown that high concentration (250 μg/ml) of Egb761 inhibited EPSCs, while low concentration (50 μg/ml) of Egb761 promoted EPSCs (Fig. 2A,B). Moreover, paired-pulse ratio (PPR, $EPSC_2/EPSC_1$) of evoked EPSCs was recorded at interpulse intervals of 25, 50, 100, 200 and 500 ms (Fig. 2C,D). Paired-samples *t* test revealed that 250 μg/ml of Egb761 incubated 20 min did not change the PPR. In order to further distinguish the presynaptic or postsynaptic effects of Egb761 on miniature EPSCs were recorded. The results showed that Egb761 inhibited mEPSC amplitude, but not the mEPSC frequency (Fig. 2E-H). Taken together, the above results

suggest that high concentration of Egb761 attenuates the LTP by inhibiting the EPSCs via postsynaptic mechanisms.

Effects of GA on TBS-LTP, evoked EPSCs and PPR on rat hippocampal CA1 neurons

As Egb761 has complex compositions, one monomer gradient ginkgolides A (GA) was selected to investigate pharmacological effects of Egb761. The results showed that the TBS-induced LTP was inhibited by GA administered 30 min before TBS stimulation (Fig. 3 AB). In addition, GA has a dose-dependent inhibitory effects on the evoked EPSC amplitudes (Fig. 3 CD), whereas the EPSC PPR was not affected by the GA (Fig. 3 EF). Taken together, the above results suggest that GA attenuates the LTP by inhibiting the EPSCs via postsynaptic mechanisms.

Effects of GB on TBS-LTP, evoked EPSCs and PPR on rat hippocampal CA1 neurons

Effects of GB, another Egb761 monomer ingredients, on LTP was investigated. The results showed that the TBS-induced LTP was inhibited by GB administered 30 min before TBS stimulation (Fig. 4 AB). In addition, GB has a dose-dependent inhibitory effects on the evoked EPSC amplitudes (Fig. 4 CD), whereas the EPSC PPR was not affected by the GB (Fig. 4 EF). The above results suggest that GB attenuates the LTP by inhibiting the EPSCs via postsynaptic mechanisms.

Effects of GC on TBS-LTP, evoked EPSCs and PPR on rat hippocampal CA1 neurons

Similar with GA and GB, Egb761 monomer ingredient GC administered 30 min before could inhibit the TBS-induced LTP (Fig. 5 AB). GC also has a dose-dependent inhibitory effects on the evoked EPSC amplitudes (Fig. 5 CD), but not the EPSC PPR (Fig. 5 EF).

Egb761, GA, GB and GC inhibited OGD-induced i-LTP in hippocampal CA1 neurous

Oxygen-Glucose-Deprivation stimulation (OGD) was used to induce i-LTP at CA1 neurons. Hippocampal slices were incubated with OGD-ACSF for 6-10min and then restored in ACSF for about 60 min to establish cerebral ischemia model. Evoked EPSCs were recorded throughout the process. The amplitude of EPSCs in the last 10 min of the recording after OGD were averaged and divided by amplitude of baseline. The results showed that EPSC amplitude increased in OGD group and formed an i-LTP, while high concentrations of Egb761, GA, GB and GC administered 30 min prior to OGD could inhibit the OGD-induced increase of EPSC amplitude (Fig. 6 AB).

Effects of Egb761, GA, GB and GC on post-synaptic GluA1 expression and phosphorylation in i-LTP

As Ser831 phosphorylation of GluA1 can increase AMPA receptor ion channel conductance, GluA1 phosphorylation at Ser831 was detected after i-LTP and effects of Egb761 and its monomer GA, GB and GC on Ser831 phosphorylation of GluA1 were studied. Hippocampal slices were incubated by ACSF containing Egb761, GA, GB and GC 30min before OGD treatment, then the slices were collected 50 min after OGD. Protein expression and Ser831 phosphorylation of GluA1 in total tissue and TIF fraction were

prepared and homogenized for immunoblotting. The OGD treatment did not affect the GluA1 protein expression in total tissue. However, the OGD treatment led to increase of GluA1protein expression in TIF, and the increase was abolished by Egb761, GA, GB and GC(Fig. 7 AB). Similarly, Ser831 phosphorylation of GluA1 increased in TIF but not in total tissue, and the increase could be abolished by Egb761, GA, GB and GC (Fig. 7 CD).

Discussion

Ginkgo is known as plant veterans and natural living fossil and Ginkgo biloba has high medicinal value. Ginkgo biloba extracts, for example Egb761, are widely used in the treatment of respiratory diseases, cardiovascular diseases, neurological diseases and so on (Guan et al. 2018; Lu et al. 2014). In the present study, we detected the attenuation effects of Ginkgo biloba extract Egb761 and its monomer component GA, GB and GC on the TBS-induced LTP recorded on rat hippocampal CA1 pyramidal neurons in acute separated brain slices. We also found that high concentrations of Egb761its monomer component GA, GB and GC suppressed the EPSC amplitude but not the paired-pulse ratio. Egb761 and its monomer components could also significantly inhibit the i-LTP in brain slice OGD model. Moreover, Egb761, GA, GB and GC attenuated the OGD-induced protein expression and Ser831 phosphorylation of GluA1 at postsynaptic membrane.

LTP in hippocampal CA1 neurons is the most classic experimental model of synaptic plasticity and its molecular mechanism was similar to long-term memory (Kerchner and Nicoll 2008). There are two main laboratory protocols to induce LTP, electric stimulation and chemical induction. For example, electric protocol TBS has been given on isolated rat hippocampal slices shaffer collateral, one kind of the wave rhythm of hippocampal CA3 brain learns which emitted high-frequency electrical stimulation can induce LTP in CA1 region (Larson and Munkácsy 2015). It has been reported that Egb761 could attenuate free radical damage, modulate cognitive impairment and dementia, prevent ischemic neuronal damage and seizure-induced neuronal damage and protect brain function (Schneider et al. 2009; Singh et al. 2019). In this article we found that Egb761 could inhibit the LTP generation and maintenance (Fig. 1). LTP are related to activation of inotrophic glutamate receptors, especially activation of NMDA receptors and trafficking of AMPA receptors (Li et al. 2019). It is confirmed that GluA1 subunit containing AMPAR aggregation at postsynaptic membrane contributes the maintenance of LTP (Mazzocchetti et al. 2020). So whether the effects of Egb761 on LTP is related to glutamate receptors? In this study we found that high concentration of Egb761 could attenuate the amplitude of evoked EPSCs on hippocampal slices (Fig. 2 AB). What's more, mEPSCs recording results shown that Egb761 could inhibit the mEPSC amplitude, but not the mEPSC frequency (Fig. 2E-H). The results suggest that Egb761 might mainly act on postsynaptic glutamate receptors, which was further proved by paired-pulse ratio (PPR) results, as the PPR had no significant change after incubation of Egb761(Fig. 2 CD).

However, in this article we found that Egb761 has bilateral adjustment effects on EPSCs (Fig. 2 AB). High concentration of Egb761, and its monomer GA, GB and GC, inhibited EPSC amplitude while low concentration of Egb761 promoted EPSC amplitude but not the paired-pulse ratio. The results suggest

that ginkgolides might act on the glutamate receptor ion channel directly, and which might be responsible for the inhibition effects of Ginkgo biloba extract on of LTP. Except for ginkgolides, Egb761 have several other gradients including quercetin (Budeč et al. 2019), which might be responsible for the promotion effects of low concentration of Egb71 on EPSCs. Moghbelinejad reported that quercetin (100 mg/kg) administered 7 days before significantly enhanced the gene expression of GluR1 subunit and GluN2 subunits in mouse hippocampus (Moghbelinejad et al. 2016). However, it still could not explain our results, that is acutely administration of low concentration Egb761 facilitated EPSCs. There are still a lot of work to do to investigate the pharmacological effects of the other monomer components of Egb761.

Currently Egb761 is widely used in prevention and treatment of cerebral vascular disease, especially ischemic stroke (Zhou et al. 2017). So we used OGD-treated brain slices to build an i-LTP experiment model. The results shown that OGD treatment induced increase in EPSCs could be inhibited by Egb761and its monomer component GA, GB and GC. LTP and i-LTP are typical research models of learning and memory and ischemic stroke. In the LTP and i-LTP process, NMDA receptors are activated and Ca^{2+} influx are boosted, which triggered a series of biochemical cascade through CaMK□. CaMK□ further catalyzes the phosphorylation of many proteins, for example GluR1 Ser831 phosphorylation and Stargazin Ser9 proteins, which play a vital role in the regulation of GluA1 trafficking (Opazo et al. 2010).

It has been reported that glutamate receptor trafficking is involved in ischemic/reperfusion injury. Mazzocchetti reported that OGD led to increase in hippocampal GluA1 but not GluA2 expression, whereas in U251-MG cells OGD induced degradation of GluA1 and GluA2 AMPAR subunits (Achzet et al. 2021; Mazzocchetti et al. 2020). As both phosphorylation and postsynaptic trafficking of GluA1 can enhance the glutamate receptor conductance, effects of Egb761, GA, GB and GC on GluA1 Ser831 phosphorylation and protein expression were detected. The results shown that i-LTP-induced increase in GluA1 expression and Ser831 phosphorylation in postsynaptic membrane but not in total homogenates were blocked by ginkgolides, which indicates that ginkgolides inhibited the activation and trafficking of postsynaptic GluA1 but not the total expression of GluA1.

Taken together, our results suggest that high concentration of Egb761 attenuated the i-LTP and postsynaptic GluA1 aggregation and phosphorylation, and the inhibition effects might act through its monomer component GA, GB and GC, which inhibited the postsynaptic ionotrophic glutamate receptors directly.

Declarations

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Figures

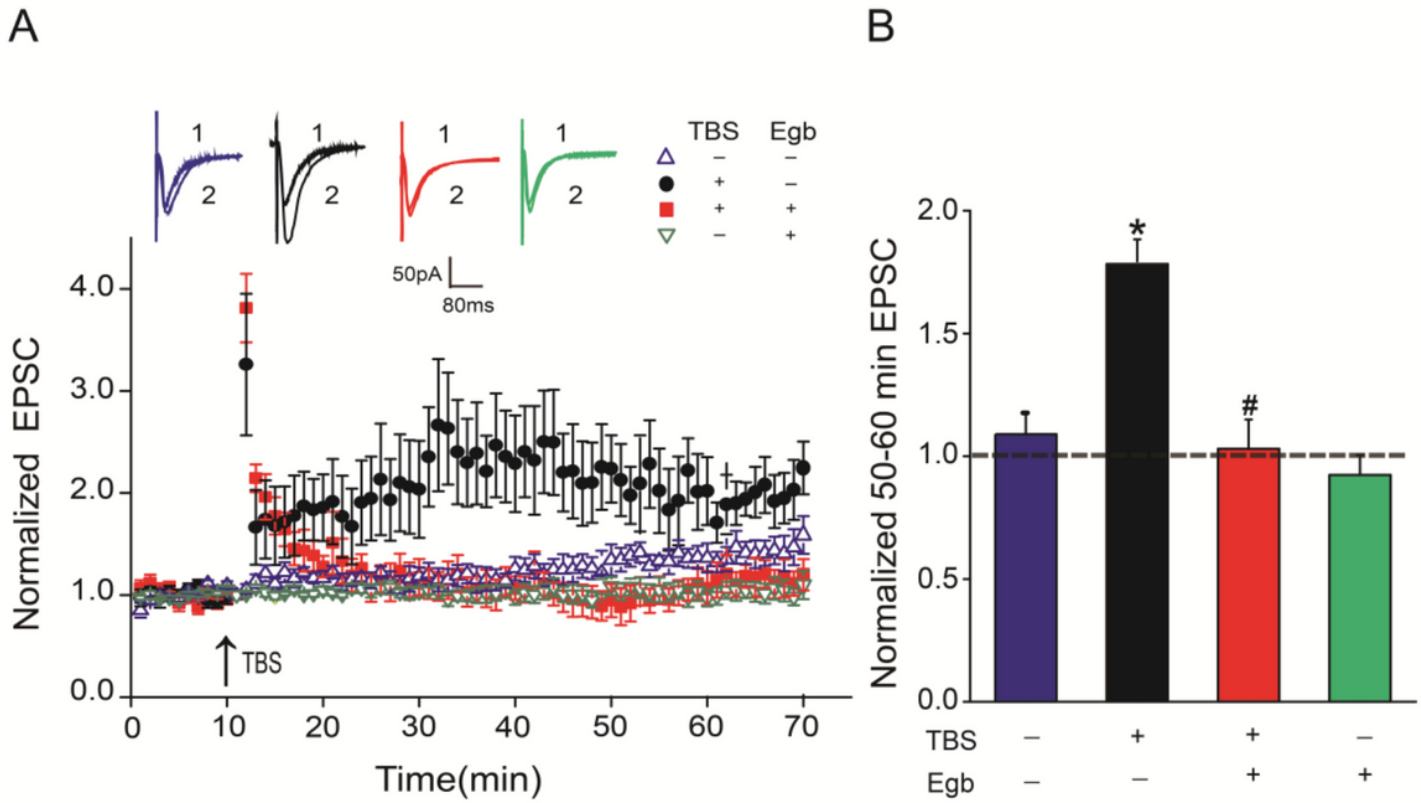

Figure 1

Egb761 attenuated TBS-induced LTP in hippocampal CA1 neurous. (A) Egb761 (250 μg/ml) inhibited TBS-induced LTP. Hippocampal slices were incubated with Egb761 (250μg/ml) more than 30 min before TBS. Representative EPSCs traces during baseline period and 50-60 min after TBS were shown for each condition above the bar graph (scale bars: 80 ms/50 pA). (B) Normalized EPSCs amplitude at 50-60 min after TBS. Egb761 inhibited TBS-induced increase of EPSCs amplitude at 50-60 min after TBS. (*P < 0.05

compared with baseline; paired t-test. #P < 0.05 compared with TBS group; one-way ANOVA followed by Dunnet t test; n = 7.)

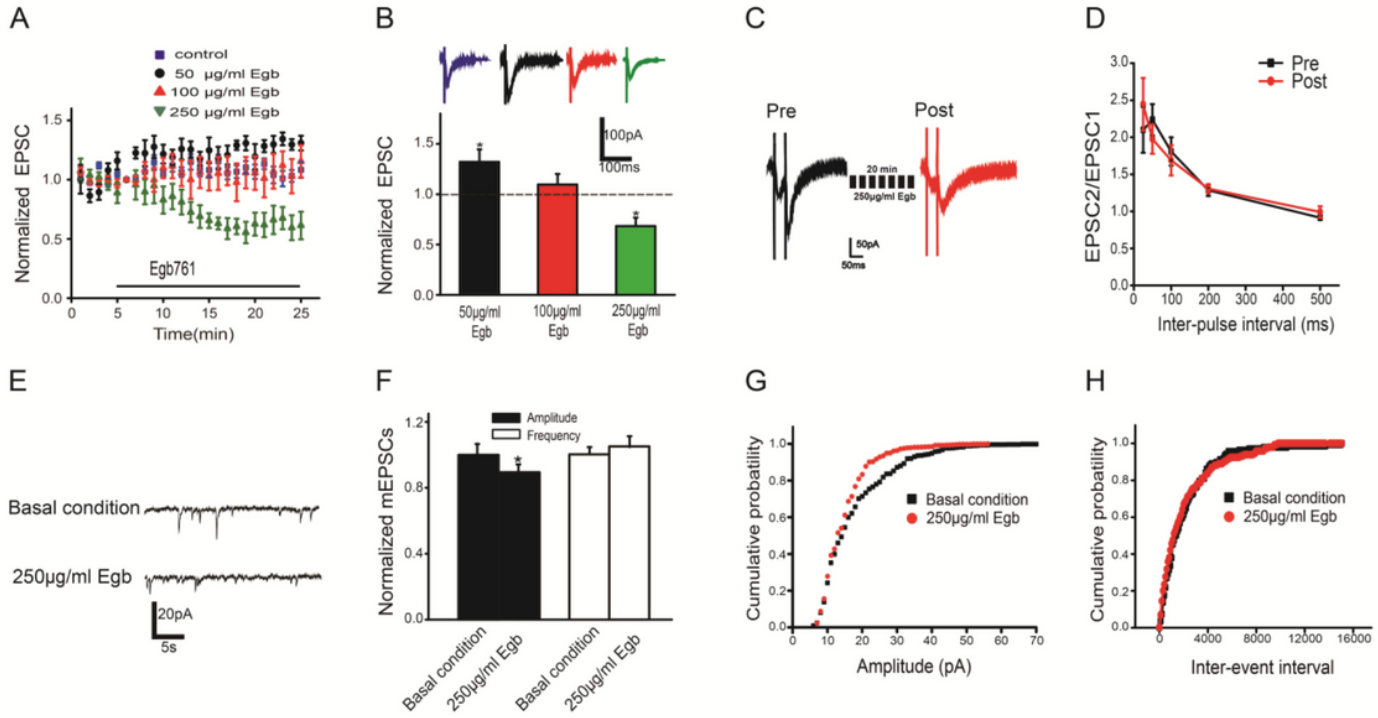

Figure 2

Biphasic effects of Egb761 on evoked EPSCs on rat hippocampal CA1 neurons. (A) Effects of different concentrations of Egb761 on the evoked EPSCs on rat hippocampal CA1 neurons. Evoked EPSCs were recorded for at least 10 min before perfusion of Egb761 to detect the baseline control. (B) Representative EPSC traces during baseline period and 20–25 min were shown above the bar graph (scale bars:100ms/100pA). Low concentration of Egb761 increased the EPSC amplitude but high concentration played an inhibition effects. (*P<0.05 compared with baseline; paired t-test, n=6). (C) Representative traces of paired-pulse EPSCs recorded at 50 ms interpulse intervals. (D) The paired-pulse ratio (PPR= EPSC2/EPSC1) at different interpulse intervals was not significantly changed by incubation of 250μg/ml Egb761 (P>0.05, n=7). (E) Sample traces of mEPSCs under basal condition and 40 min after incubation of Egb761 (250 μg/ml). (F) Statistic results of the mEPSCs amplitude and frequency. Egb761 inhibited the mEPSC amplitudes but not the mEPSC frequency (*P<0.05 compared with basal condition, paired t-test, n=7). (G-H) Cumulative results of mEPSC amplitudes and inter-event intervals from a typical neuron.

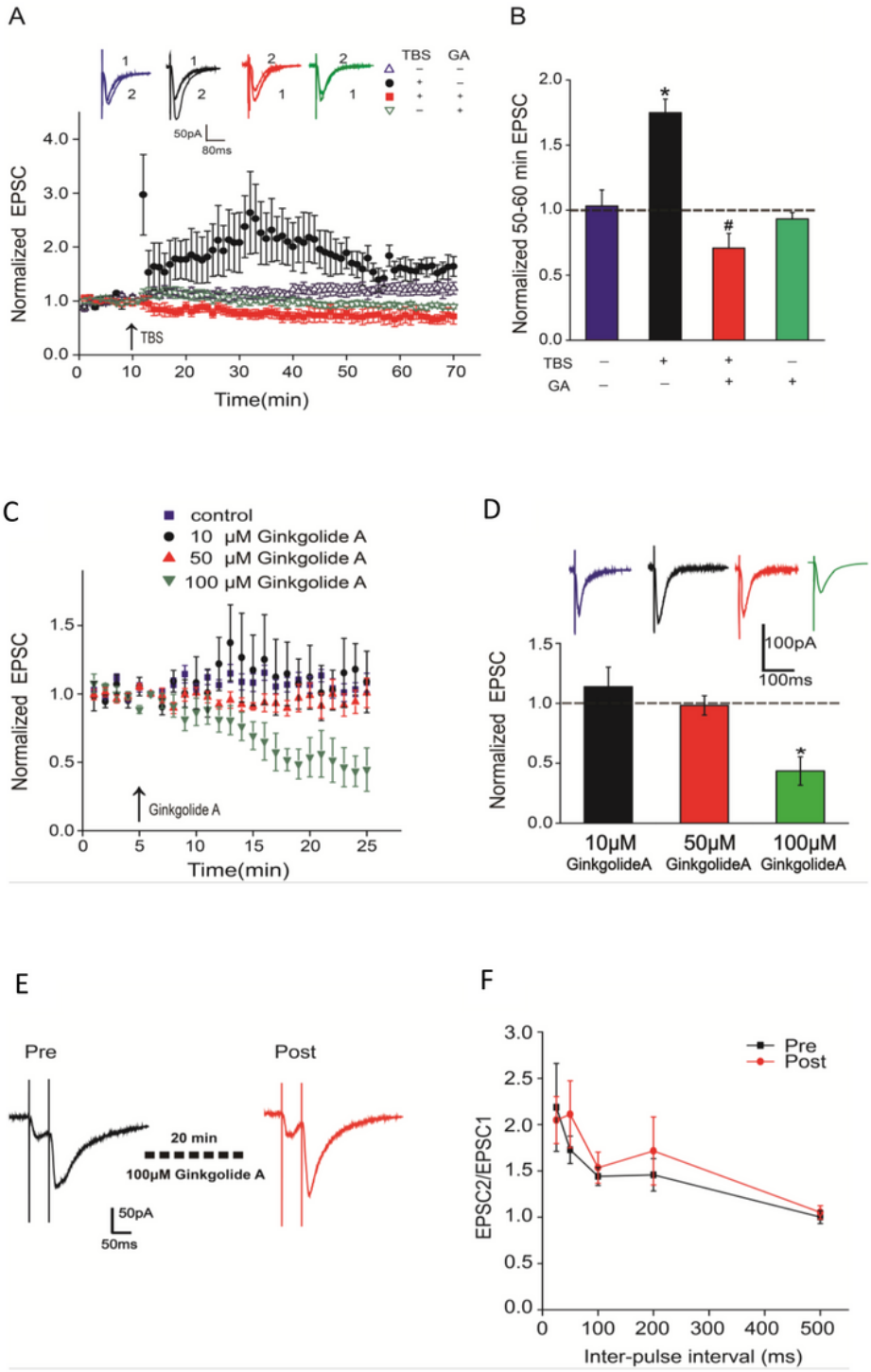

Figure 3

GA blocked TBS-induced LTP in hippocampal CA1 neurons. (A) GA significantly attenuated TBS-induced LTP. Hippocampal slices were incubated with GA (100μM) more than 30 min before TBS. Representative EPSCs traces during baseline period and 50-60 min after TBS were shown for each condition above the bar graph (scale bars: 80 ms/50 pA). (B) Statistic results of GA inhibition effects on TBS-induced increase of EPSCs amplitude at 50-60 min. (*P < 0.05 compared with baseline, paired t-test. #P < 0.05

compared with TBS group; one-way ANOVA followed by Dunnet t test; n=6). (C) Effects of different concentrations of GA on the evoked EPSCs on rat hippocampal CA1 neurons. Evoked EPSCs were recorded for at least 10 min before perfusion of GA to detect the baseline control. (D) Representative EPSC traces during baseline period and 20-25 min were shown above the bar graph (scale bars:100ms/100pA). EPSC amplitude decreased after incubation of GA. (*P<0.05 compared with baseline; paired t-test; n=6). (E) Representative traces of paired-pulse EPSCs recorded at 50 ms interpulse intervals. (F) GA (100μM) had no effect on the paired-pulse ratio (PPR= EPSC2/ EPSC1) (P>0.05, n=6).

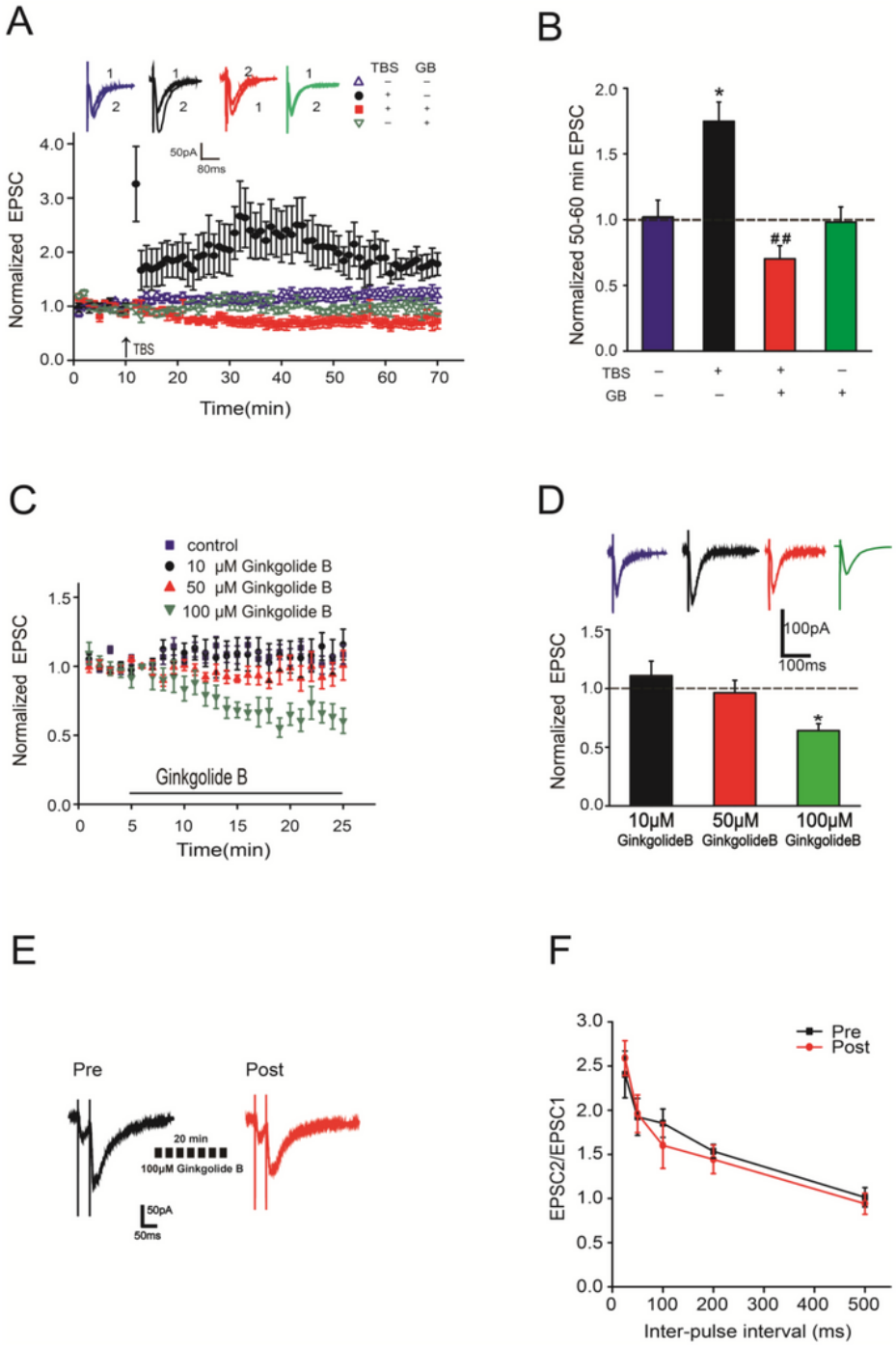

Figure 4

GB blocked TBS-induced LTP in hippocampal CA1 neurons. (A) GB significantly attenuated TBS-induced LTP. Hippocampal slices were incubated with GB (100μM) more than 30 min before TBS. Representative EPSCs traces during baseline period and 50-60 min after TBS were shown for each condition above the bar graph (scale bars: 80 ms/50 pA). (B) GB inhibited TBS-induced increase of EPSCs amplitude at 50-60 min. (*P < 0.05 compared with baseline, paired t-test. #P < 0.05 compared with TBS group; one-way ANOVA followed by Dunnet t test; n=6). (C) Effects of different concentrations of GB on the evoked EPSCs on rat hippocampal CA1 neurons. Evoked EPSCs were recorded for at least 10 min before perfusion of GB to detect the baseline control. (D) Representative EPSC traces during baseline period and 20-25 min were shown above the bar graph (scale bars:100ms/100pA). EPSC amplitude decreased after incubation of GB. (*P<0.05 compared with baseline; paired t-test; n=6). (E) Representative traces of paired-pulse EPSCs recorded at 50 ms interpulse intervals. (F) GB (100μM) had no effect on the paired-pulse ratio (PPR= EPSC2/ EPSC1) (P>0.05, n=6).

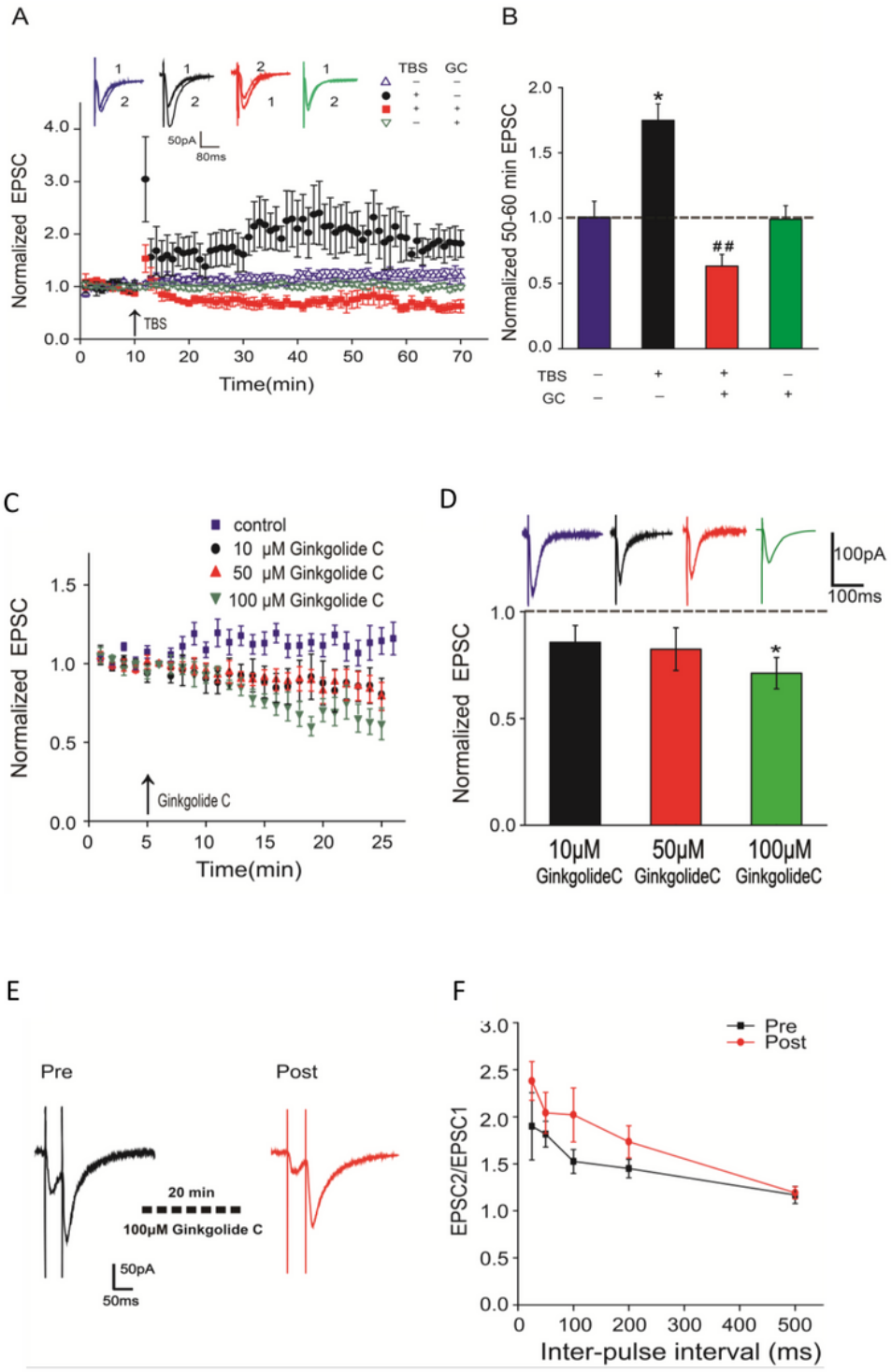

Figure 5

GC blocked TBS-induced LTP in hippocampal CA1 neurons. (A) GC significantly attenuated TBS-induced LTP. Hippocampal slices were incubated with GC (100μM) more than 30 min before TBS. Representative EPSCs traces during baseline period and 50-60 min after TBS were shown for each condition above the bar graph (scale bars: 80 ms/50 pA). (B) Statistic results of GC inhibition effects on TBS-induced increase of EPSCs amplitude at 50-60 min. (*P < 0.05 compared with baseline, paired t-test. #P < 0.05

compared with TBS group; one-way ANOVA followed by Dunnet t test; n=6). (C-D) Effects of different concentrations of GC on the evoked EPSCs on rat hippocampal CA1 neurons. Evoked EPSCs were recorded for at least 10 min before perfusion of GC to detect the baseline control. Representative EPSC traces during baseline period and 20-25 min were shown above the bar graph (scale bars:100ms/100pA). EPSC amplitude decreased after incubation of GC. (*P<0.05 compared with baseline; paired t-test; n=6). (E-F) GC (100μM) had no effect on the paired-pulse ratio (PPR= EPSC2/ EPSC1) (P>0.05, n=6).

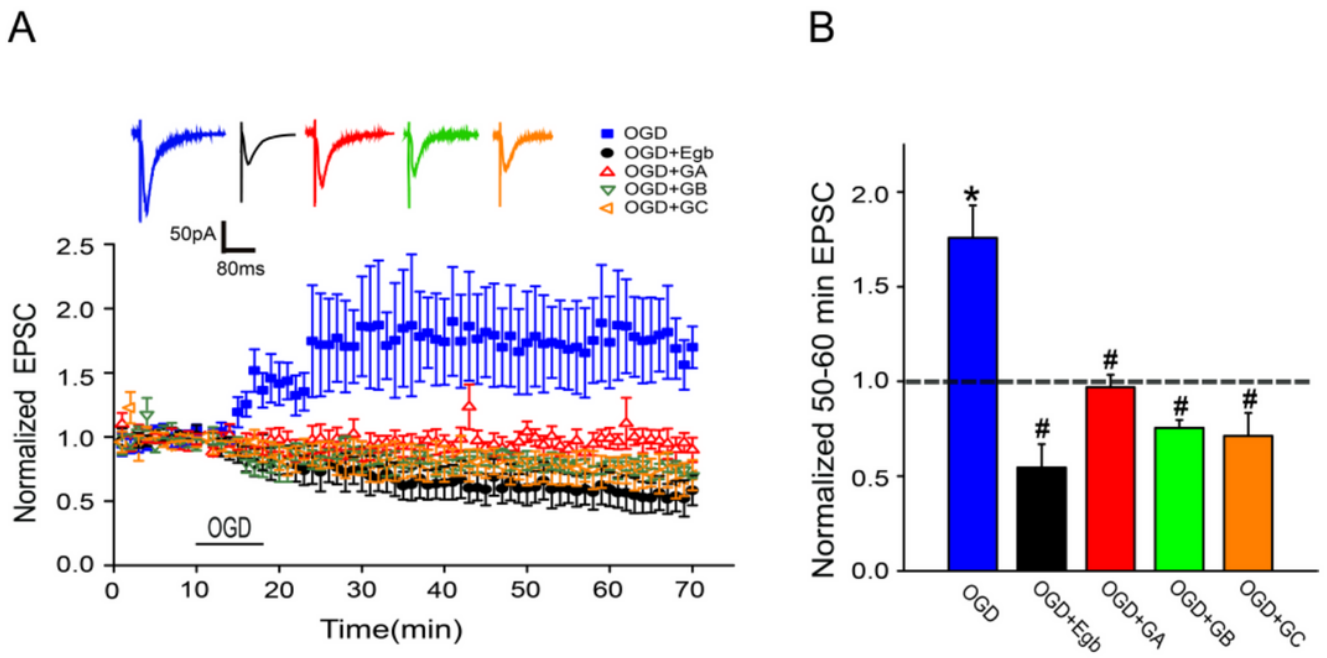

Figure 6

Egb761, GA, GB and GC inhibited the i-LTP. (A) Pathological i-LTP of AMPAR mediated EPSCs induced by brief oxygen and glucose deprivation. The Pathological i-LTP was abolished by Egb761 (250μg/ml), GA, GB and GC (100μM). (B) Summary of data showing the effects of Egb761, GA, GB and GC on i-LTP. (*P < 0.05 compared with baseline; #P < 0.05 compared with OGD group; one-way ANOVA followed by LSD test; n = 6).

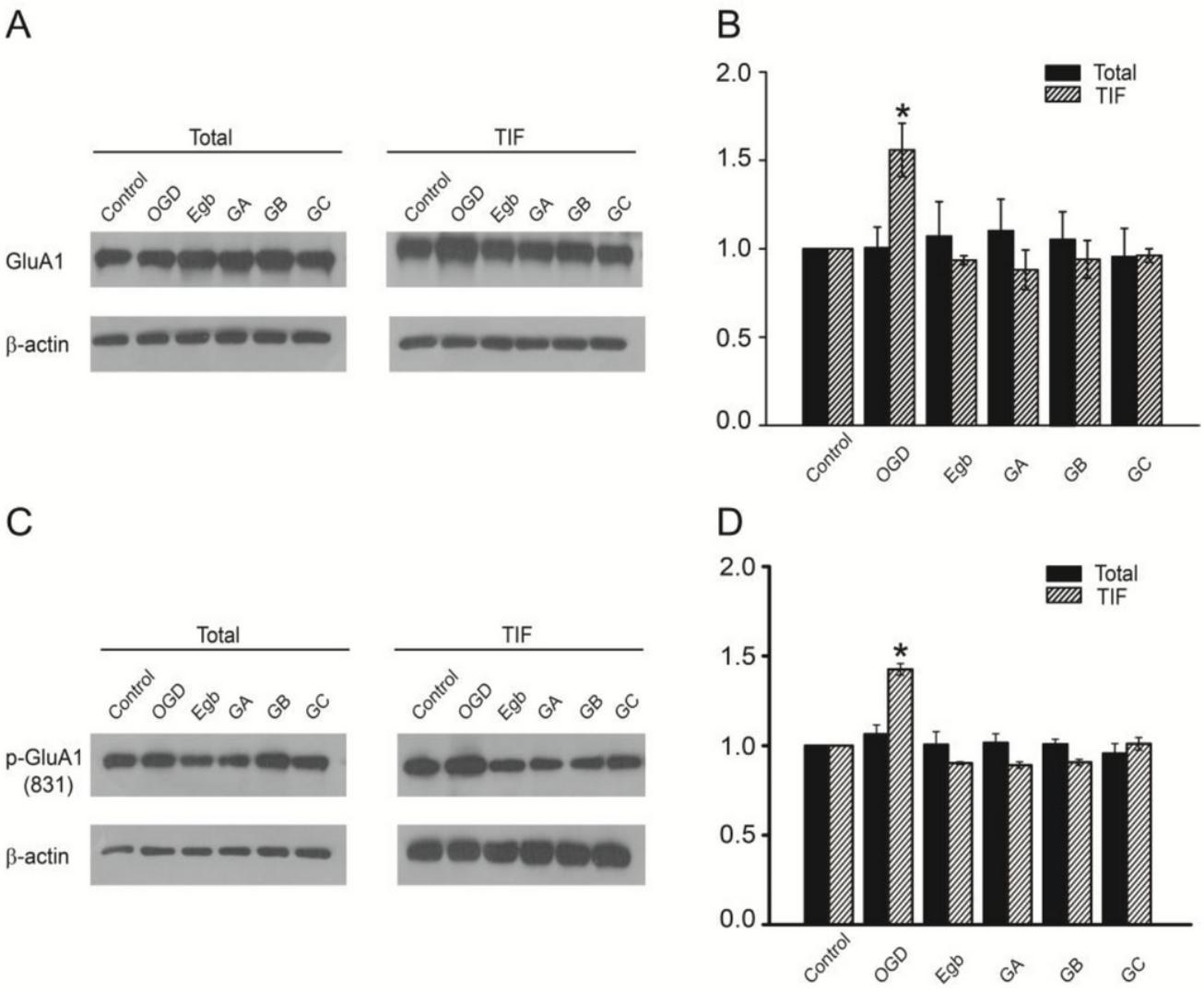

Figure 7

Egb761 and its monomer components inhibited the GluA1 trafficking to synapses in i-LTP. (A) GluA1 expression in Total and TIF were detected by western blots. (B) Statistic results of GluA1 expression. Total GluA1 did not change in all groups, whereas TIF GluA1 expression was significant increased by OGD. The increase was attenuated by Egb761, GA, GB and GC (*P<0.05 compared with group of control; one-way ANOVA followed by LSD test; n=3). (C) The phosphorylation of GluA1 (Ser831) in Total and TIF were detected by immunoblotting. (D) Statistic results of the phosphorylation of GluA1 (Ser831). Total GluA1 (Ser831) phosphorylation did not change after OGD. However, OGD induced increase of GluA1 (Ser831) phosphrylation were blocked by Egb761, GA, GB and GC (*P< 0.05 compared with group of control; #P < 0.05 compared with OGD group; n=3.)