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Isoliquiritigenin, a Chalcone Compound, Enhances Spontaneous Inhibitory Postsynaptic Response

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Isoliquiritigenin (ILTG) is a chalcone compound and shows various pharmacological properties, including antioxidant and anti-inflammatory activities. In recent study, we have reported a novel role of ILTG in sleep through a positive allosteric modulation of gamma-aminobutyric acid type A ($GABA_A$)-benzodiazepine (BZD) receptors. However, the effect of ILTG in $GABA_A$R-mediated synaptic response in brain has not been tested yet. Here we report that ILTG significantly prolonged the decay of spontaneous inhibitory postsynaptic currents (sIPSCs) mediated by $GABA_A$R in mouse hippocampal CA1 pyramidal neurons without affecting amplitude and frequency of sIPSCs. This enhancement was fully inhibited by flumazenil (FLU), a specific $GABA_A$-BZD receptor antagonist. These results suggest a potential role of ILTG as a modulator of GABAergic synaptic transmission.

Key words: Isoliquiritigenin, $GABA_A$-BZD receptor, sIPSC

INTRODUCTION

Isoliquiritigenin (ILTG, 2′,4,4′-trihydroxychalcone) is a chalcone compound and found in various flavonoids such as *Glycyrrhiza uralensis* (licorice), *Allium ascalonicum, Sinofranchetia chinensis, Dalbergia odorifera*, and *Glycine max* L. [1-5]. It has been reported that ILTG has various pharmacological properties including anti-inflammatory, antioxidant, anticancer, anti-angiogenic, and anti-allergic [5-9]. In addition to these properties, it has been demonstrated that ILTG has some neurological functions such as inhibition of cocaine-induced dopamine release and hypnotic effect by modulating gamma-aminobutyric acid receptors (GABARs) [10, 11]. However, the exact effect of ILTG in inhibitory synaptic activity and type of GABA receptor have not been demonstrated yet.

In the present study, we investigated the modulation of GABAergic synaptic response by ILTG in mouse hippocampal CA1 pyramidal cell using whole-cell patch clamp technique. We found that ILTG specifically enhanced the decay tau of sIPSC by modulating $GABA_A$-BZD receptor. However, the amplitude and frequency of sIPSC were not affected by ILTG. Therefore, our results suggest a potential role of ILTG as a modulator of GABAergic synaptic transmission.

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MATERIALS AND METHODS

Slice preparation

Adult mice (7~9 weeks) were deeply anaesthetized until cessation of breathing and subsequently decapitated. The brain was rapidly removed and submerged in an ice-cold oxygenated artificial cerebrospinal fluid (ACSF) composed of (in mM) 130 NaCl, 24 NaHCO$_3$, 3.5 KCl, 1.25 NaH$_2$PO$_4$, 1 CaCl2, 3 MgCl$_2$, 10 glucose at pH 7.4, and was bubbled with 5% CO$_2$ / 95% O$_2$. Transverse mouse brain slices (350~400 μm) containing hippocampus were acutely prepared with a Leica vibratome (Leica VT1000S), and incubated in a chamber with oxygenated ACSF at room temperature for 1 hr before use.

Recording of sIPSCs

The standard ACSF recording solution was composed of (mM): 130 NaCl, 24 NaHCO$_3$, 3.5 KCl, 1.25 NaH$_2$PO$_4$, 1.5 CaCl$_2$, 1.5 MgCl$_2$ and 10 glucose saturated with 95% O2~5% CO2, at pH 7.4. The internal solution was composed of (mM): 140 CsCl, 10 EGTA, 10 HEPES, 4 Mg-ATP, 2 QX-314. To block the spontaneous EPSC, APV (50 μM; Tocris) and CNQX (20 μM; Tocris) were added into ACSF. Recordings were obtained using Axopatch 200A (Axon instruments, Union City, CA, USA) and filtered at 2 kHz. In case of sIPSC recording, recordings were digitized at 10 kHz, and analyzed using pCLAMP 9 (Molecular devices) and Mini Analysis Program (Synaptosoft) as previously described [12]. The sEPSCs were automatically detected and grouped as fast (1~5 ms) and slow rise time (5~10 ms). All experimental procedures described were performed in accordance with the institutional guidelines of
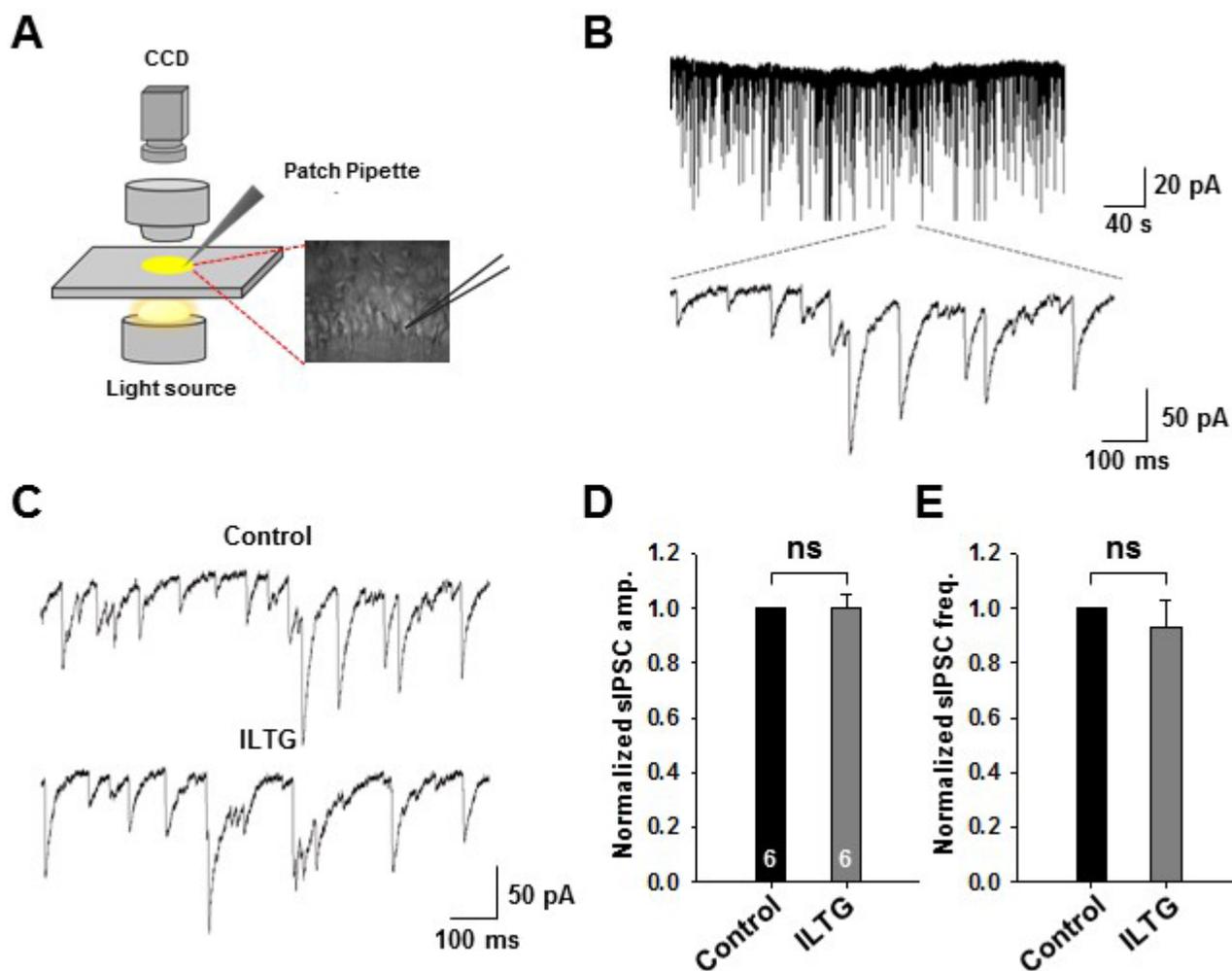
Fig. 1. ILTG does not affect amplitude and frequency of sIPSCs. (A) Schematic showing slice patch clamp. (B) Representative whole trace (upper) and magnified trace (lower) of sIPSC. (C) Representative trace of sIPSC before and after treatment of ILTG. (D and E) Summary bar graphs of the amplitude (D) and frequency (E) of sIPSC after normalization by control response (Students' tailed t-test). Data are represented as mean +/− SEM. ns indicates nonsignificant difference p>0.05, n=6.

Korea Institute of Science and Technology (KIST, Seoul, Korea).

Statistical analyses

Statistical comparisons were performed using independent t-tests for two groups and one-way ANOVA test for three groups. Data were expressed as the mean±S.E.M. Differences were considered significant at **$p < 0.01$.

RESULTS

ILTG does not affect amplitude and frequency of sIPSCs

To examine the role of ILTG in the inhibitory synaptic response, we performed the whole-cell patch clamp in hippocampal CA1 pyramidal neurons (Fig. 1A) and measured the sIPSCs at -60mV in the presence of APV and CNQX to block the EPSCs (Fig. 1B). However, the amplitude and frequency of sIPSC were not changed before and after treatment of ILTG (1 μM) by bath application (Fig. 1C~E). This indicates that ILTG does not affect the presynaptic release of GABA and postsynaptic receptor number.

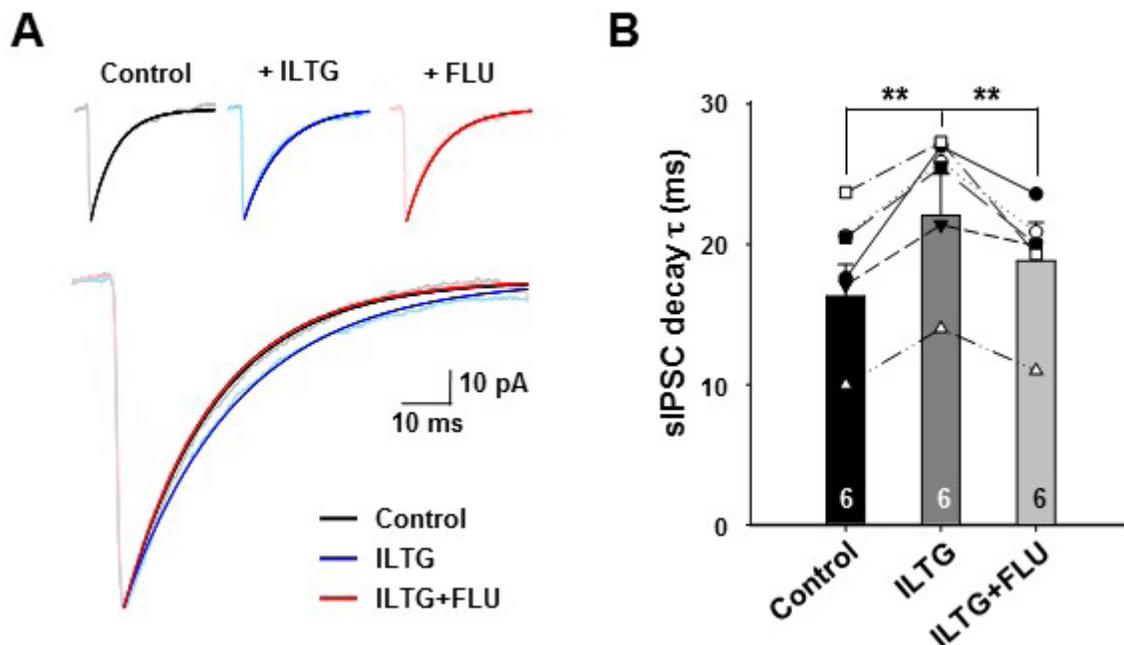### ILTG significantly prolonged the sIPSC decay

Although ILTG had little effect on sIPSC amplitude and frequency, we analyzed the sIPSC in more detail by measuring the decay tau value after single-exponential decay fitting. Interestingly, ILTG significantly increased the decay time (Fig. 2 control:

18.3±1.9 ms; ILTG: 23.5±2.1 ms). In a recent study, it has been reported that ILTG enhanced the GABA-induced current in dorsal raphe neurons by modulating GABA$_A$R [11]. To test this, we applied flumazenil (FLU, 5 μM), a specific GABA$_A$R antagonist, into ILTG-treated slice. The enhancement of decay time of sIPSC by ILTG was fully restored to control (Fig. 2B, control: 18.3±1.9 ms; ILTG: 23.5±2.1 ms; ILTG+FLU: 19.1±1.7 ms).

We have reported that ILTG functions as a positive allosteric modulator of GABA$_A$ –BZP receptor [11]. To confirm this, we measured the decay time of sIPSC using well known modulator for GABA$_A$ –BZP receptor, diazepam (DZP, 1 μM). Just as ILTG, DZP significantly enhanced the decay time of sIPSC (Fig. 3A and B, control: 19.2±1.3 ms; ILTG: 24.9±2.1.6 ms), but did not affect the amplitude and frequency of sIPSC (Fig. 3C and D). These results suggest that ILTG prolonged the sIPSC decay effectively by modulating the GABA$_A$ –BZP receptor via a mechanism similar to that of DZP.

DISCUSSION

Here we reported that ILTG significantly enhanced the decay time of sIPSC but had little effect on sIPSC amplitude and frequency. These results is consistent with other modulator for GABA$_A$ –BZP receptor, flunitrazepam [13] and DZP (Fig. 3). These results suggest that a similar number of GABA channels

Fig. 2. ILTG significantly prolonged the sIPSC decay. (A) Averaged sIPSCs after normalization by peak. Decay was fitted to one-exponential functions. Upper: individual traces of each conditions. Lower: Superimposed traces. (B) Summary bar graph of sIPSC decay time. Symbols represent individual neurons. Data are represented as mean +/− SEM. **$p < 0.01$, one-way ANOVA, n=6.
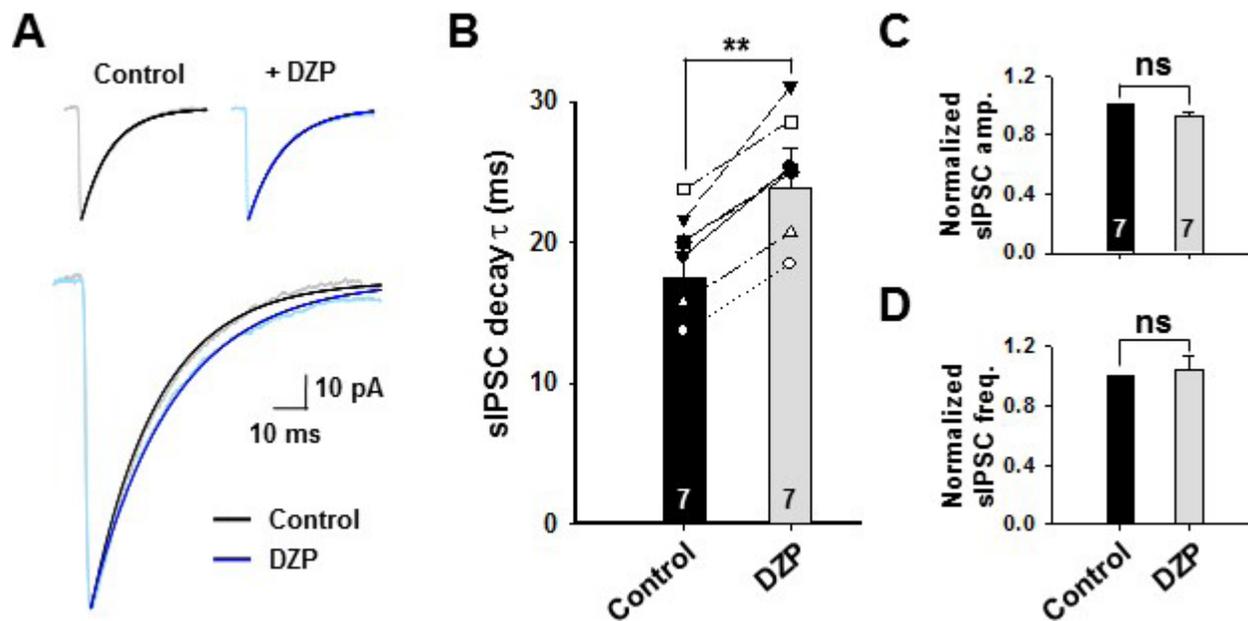
Fig. 3. DZP significantly prolonged the sIPSC decay but not amplitude and frequency of sIPSC. (A) Averaged sIPSCs after normalization by peak. Decay was fitted to one-exponential functions. Upper: individual traces of each conditions. (B) Summary bar graph of sIPSC decay time. Symbols represent individual neurons. (C and D) Summary bar graphs of the amplitude (C) and frequency (D) of sIPSC after normalization by control response. **$p<0.01$, Students' tailed t-test. ns indicates nonsignificant difference $p>0.05$, n=7.

are activated initially during the brief synaptic release of GABA generating IPSC. However, the channel opening frequency is increased by modulators for $GABA_A$ –BZP receptor producing longer decays after channel activation [14, 15]. In a previous study, we showed that ILTG enhanced the GABA-induced current in a dose-dependent manner in dorsal raphe neurons [11]. The enhancement was 266% by DZP and 151% by ILTG at 1 μM concentration. We prediced that ILTG can enhance the decay time of sIPSCs in a concentration dependent manner, however, in the present study we used 1 μM ILTG to obtain maximal enhancement. Contrary to the GABA-induced current, the enhancement of sIPSC decay time was similar between DZP and ILTG at 1 μM concentration.

$GABA_A$Rs activity can be modulated by various drugs including benzodiazepines, barbiturates, ethanol, neurosteroids, anesthetics, and ionic zinc with separate binding sites [16-18]. This modulation was measured in various brain regions such as thalamus, hippocampus, suprachiasmatic nucleus [13, 19, 20] and had many critical role in brain activity not only in physiological condition but in pathophysiological condition including various diseases such as depression, seizure, schizophrenia, and etc [21-23].

Here we have investigated that ILTG modulates the sIPSC by enhancing response time as a positive allosteric modulator of $GABA_A$ –BZP receptor in hippocampal CA1 pyramidal neurons. It has been reported that ILTG inhibits the mitogen-activated

protein kinase (MAPK) pathway and chronic benzodiazepine administration reduced the NMDAR activation in hippocampus [8, 19]. Both MAPK pathway and NMDAR are critically involved in hippocampal synaptic plasticity and spatial memory [24-27]. We can postulate that ILTG could be a negative regulator for NMDAR-dependent synaptic plasticity and relating behavior, which has not been studied yet. Future studies are needed to study the function of ILTG in hippocampal brain function such as synaptic plasticity and memory.

DZP is widely used to treat various diseases including anxiety, seizures and insomnia by modulating $GABA_A$ Rs through the binding to the $GABA_A$ –BZP receptor. However, DZP has some adverse effects including anterograde amnesia, sedation and depression [28-30]. ILTG shows hypnotic effects, having 65 fold higher binding affinity than that of DZP [11], suggesting that ILTG could be a potential drug for the treatment of sleep as a natural compound from flavonoids. Therefore, this study suggests that ILTG is a potential drug to treat various diseases of sleep and seizures related to GABAergic synaptic transmission.

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