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Dopamine inhibits excitatory neurotransmission in basolateral amygdala during development via pre-synaptic mechanism

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Abstract

OBJECTIVES: Dopaminergic signaling in the basolateral amygdala (BLA) is important for emotion-related activity. However, little is known about the influence of dopamine (DA) on excitatory synaptic transmission of pyramidal neurons in BLA at early developmental stage. Here in this study, we observed the effect of DA on excitatory neurotransmission in the pyramidal cells of BLA in acute slices.
METHODS: Acute slices from amygdala of rats at the age of 14–16 days were prepared and maintained in vitro using standard method. Whole-cell patch clamp recordings were performed to examine the evoked excitatory postsynaptic current (eEPSC), spontaneous excitatory postsynaptic current (sEPSC) and miniature excitatory postsynaptic current (mEPSC). Drugs including DA and synaptic blockers were added in recording solution due to different experimental designs.
RESULTS: We found that bath application of DA at a concentration of 100 μM significantly inhibited the amplitude of evoked EPSC. However, the amplitude and frequency of mEPSC were not affected. We also found increased pair pulse facilitation after DA application, indicating DA inhibited excitatory neurotransmission through suppression of release probability at the pre-synaptic terminals. Importantly, DA was also effective in decreasing activity induced upregulation in sEPSCs. Moreover, the DA effects were not affected by either antagonist of dopamine 1 or dopamine 2-like receptors.
CONCLUSION: We studied the effects of DA on excitatory neurotransmission and found that DA inhibited glutamatergic synaptic transmission via modulation of pre-synaptic release probability.

ORIGINAL ARTICLE

INTRODUCTION

The basolateral amygdala (BLA) is one of the brain regions that controls emotional behaviors, including fear responses and social behaviors (Aggleton 1993). Thus it is important to study the neurotransmission within neurons in BLA to investigate how changes in the activity of neurons in BLA affect social and emotional behaviors.

Dopamine (DA) is synthesized by dopamine-containing neurons in the midbrain, mostly in the substantia nigra, ventral tegmental area, as well as the medial zona interna (Momiyama *et al.* 1996). Previous work have shown that DA is responsible for the modulation of both excitatory and inhibitory synaptic transmission (Anzalone *et al.* 2012; Cornil & Ball 2008; Diaz *et al.* 2011). Since glutamatergic synaptic transmission is one of the most important excitatory synaptic transmission in the central nervous system and plays a vital role in regulating a variety of brain functions (Zucker & Regehr 2002), numerous work was done studying the effect of DA on glutamatergic synaptic transmission in multiple brain regions, including the hippocampus, the prefrontal cortex, neucleus accumbens, basal forebrain and ventral tegmental area (Hjelmstad 2004; Ito & Schuman 2007; Koga & Momiyama 2000). These studies showed that DA is capable in regulating presynaptic release of glutamate in basal forebrain, basolateral amygdala and ventral tegment (Koga & Momiyama 2000; Momiyama *et al.* 1996; Diaz *et al.* 2011), as well as in modulating the expression of postsynaptic N-methyl-D-aspartic acid (NMDA) receptors in the hippocampus (Koga & Momiyama 2000).

Previous work had focused on how dopamine affected emotional behaviors due to strong connections between dopaminergic neurons and neurons in the BLA (Asan 1997). It has also been reported that application of DA or DA agonists facilitated amygdala-dependent fear conditioning (Borowski & Kokkinidis 1996). Previous studies have also shown that DA application increased the activity of projection neurons in the BLA in adult animals through activation of D1 receptors and NMDA receptors (Kroner *et al.* 2005; Pickel *et al.* 2006). However, the physiological roles of DA on the pyramidal neurons in the BLA during development have not been sufficiently elucidated.

Here in this paper, we studied the effects of DA application on pyramidal neurons in the BLA in developing rats. Using whole-cell patch-clamp recordings, we studied the effect of DA application on evoked excitatory postsynaptic currents (eEPSCs), spontaneous EPSCs and pair pulse facilitation (PPF). We found a different role of DA on pyramidal neurons in the BLA during development via pre-synaptic mechanism.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (SD rats, 14–16 days old) reared in 12 h light/dark schedule were used in all experiments. Food and water were available *ad libitum*. Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.). All procedures were carried out in accordance with international guidelines for the care and use of laboratory animals, which were approved by the Animal Care and Use Committee of the Three Gorges University.

Preparation of BLA slices

Basolateral amygdala slices were prepared according to previously described procedures [27]. Briefly, rat brain was quickly removed after decapitation and submerged in ice-cold artificial cerebral spinal fluid (ACSF) containing the following (in mM): 126 NaCl, 5 KCl, 2 $CaCl_2$, 2 $MgSO_4$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 10 glucose, 10 sucrose and saturated with 95% O_2/5% CO_2. A block of tissue containing BLA was placed on the cutting stage of vibratome (VT1000M/E; Leica). Serial coronal slices (300 μm) were prepared and transferred to an incubating chamber (30–32 °C) for at least 1 h recovery before recording.

Whole-cell recording

Pyramidal cells of BLA were visualized using infrared Nomarski optics. Whole-cell voltage-clamp recordings were made using patch electrodes (2–3 MΩ) containing the following (in mM): 140 K-gluconate, 0.1 $CaCl_2$, 2 $MgCl_2$, 1 EGTA, 2 ATP·K_2, 0.1 GTP·Na_3, and 10 HEPES, pH 7.4. Voltage and current signals were recorded with an Axopatch 200B amplifier (Molecular Device, USA) connected to a Digidata1200 interface (Molecular Device, USA). The data were recorded using pClamp software (version 6.0; Axon Instruments). Resting membrane potential and action potentials were recorded under the current-clamp mode. For excitatory postsynaptic current (EPSC) recordings, cells were held at –70 mV under a voltage-clamp mode. A concentric stimulating electrode (FHC, USA) was placed near the external capsule, 200–300 μm lateral to the recorded cell. Picrotoxin (50 μM) was included in ACSF to block gamma-aminobutyric acid A ($GABA_A$) receptors. Stimulation pulses were given at 0.05 Hz to evoke EPSCs with 30–40% of the maximum amplitude, usually 200 pA.

To observe paired pulses facilitation, two synaptic responses were evoked by a pair of stimuli given at 50 ms interval at 0.05 Hz. Miniature EPSCs (mEPSCs) were recorded at a holding potential of –70 mV under the voltage-clamp mode in the presence of tetrodotoxin (TTX, 0.5 μM, Research Institute of Aquatic Products of Hebei, China) and picrotoxin (50 μM) to block voltage-dependent sodium channels and $GABA_A$ receptors, respectively. Spontaneous EPSCs (sEPSCs) were recorded in the absence of TTX and presence of picrotoxin. The series resistance was monitored by measuring the instantaneous current in response to a 5 mV voltage step command. Cells with more than 15% changes of series resistance were discarded. All drugs were from Sigma unless otherwise stated.

DA was applied to neurons at BLA by aCSF circulation with 100 μM DA in the solution. Drugs including KCl, 4-Aminopyridine and dopamine receptor antagonists were performed to neurons by bath application in aCSF circulation with drugs in certain concentration.

Data and statistical analysis

Electrophysiology data were analyzed using Mini Analysis Program, SigmaPlot, and Origin. PPF was calculated by subtraction the amplitudes of the second EPSC (EPSC2) and the first EPSC (EPSC1) according to the following formula: PPF = (EPSC2–EPSC1) / EPSC1 × 100%, where EPSC1 and EPSC2 are the average amplitudes of the first and second EPSCs.

Results were expressed as mean ± SEM. Electrically evoked EPSCs, mEPSCs and sEPSCs were analyzed using students' *t*-tests for comparison of two conditions or one-way ANOVA test for three and more conditions. The cumulative distribution of amplitude and inter-event intervals of mEPSCs or sEPSCs were analyzed using Kolmogorov-Smirnov test (K-S test). The *p*-value <0.05 was considered as statistical significance. In all cases, n refers to the number of cells studied. Each experiment included at least four animals.

RESULTS

DA inhibited the amplitude of eEPSCs

We first studied the effect of DA application on evoked EPSCs. Bath application of DA (100 μM) significantly inhibited the amplitude of EPSCs. The percentage of eEPSC amplitude inhibited at 15–20 min after DA application was 54.99±4.78% (n=6, $p<0.01$, Figure 1A, B). The time course of eEPSCs after DA application showed that DA started to inhibit eEPSC amplitude 3 min after DA administration ($p<0.05$), with a maximal effect at 15 min after DA application. Such effect was reversible by washout with ACSF (Figure 1C). We also found that DA affected eEPSC amplitude in a dose-dependent manner in the range of 3–300 μM, with a plateau after 100 μM (Figure 1D).

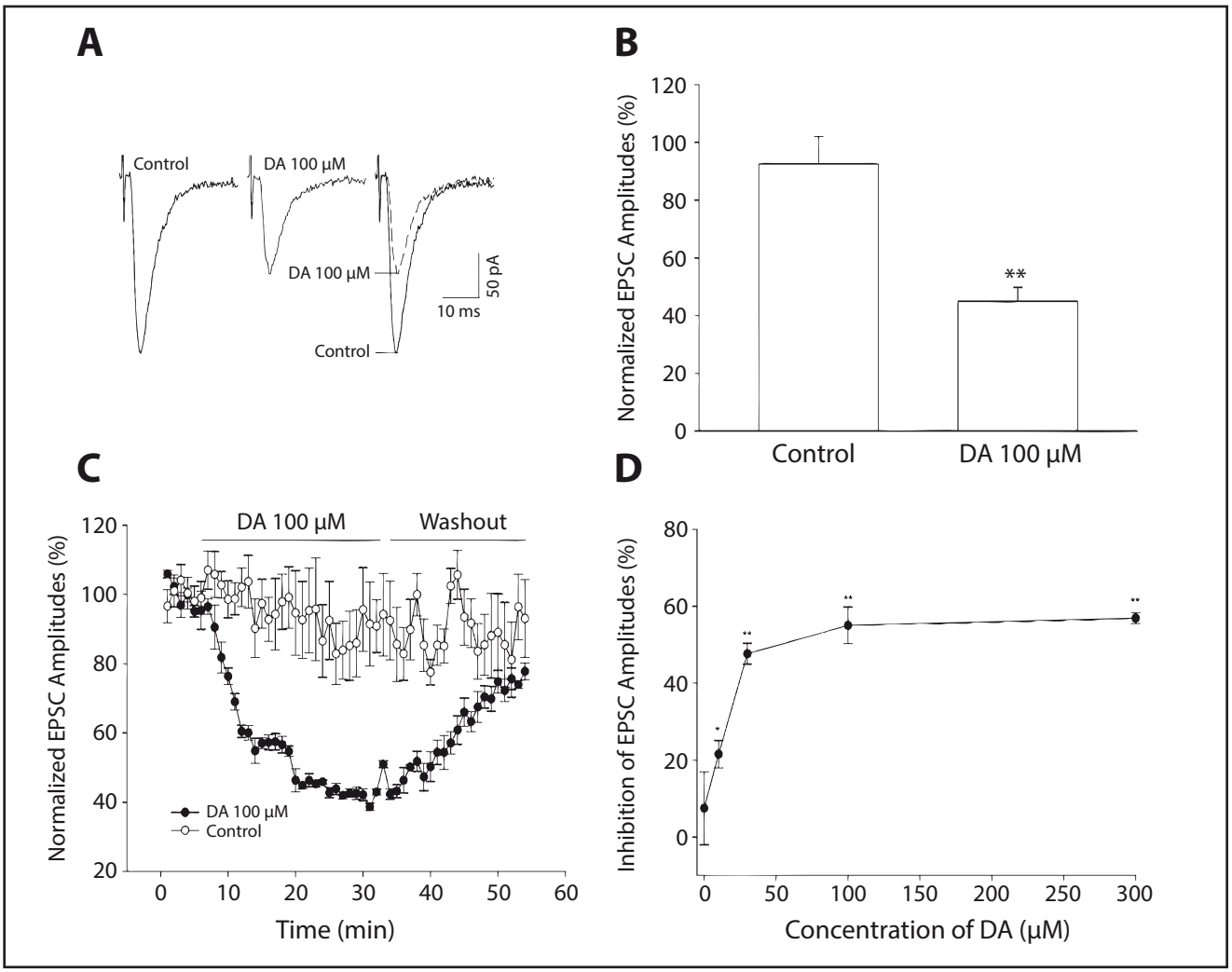

Fig. 1. The effect of DA on stimulus-evoked EPSCs in the pyramidal cells of BLA. (A) Representative traces of stimulus-evoked EPSCs in the absence and presence of DA. (B) Bar graph showing the amplitude of eEPSCs in each condition (n=6, ** $p<0.01$). (C) Time course experiment showing the effect of DA on eEPSCs (n=6). (D) Dose effect of DA on eEPSCs (n=6, ** $p<0.01$, * $p<0.05$)

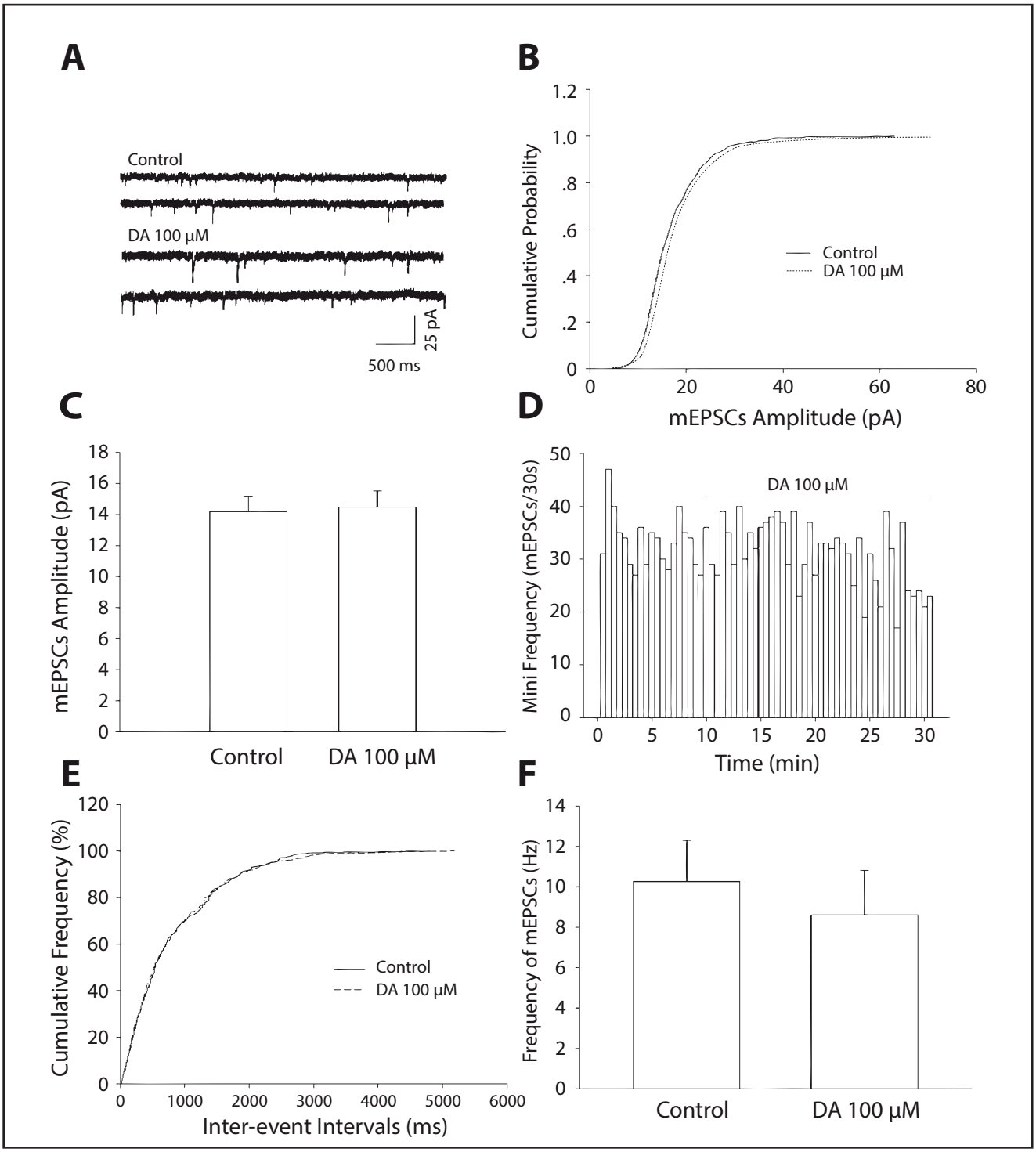

Fig. 2. The effect of DA on mEPSCs in the pyramidal cells of BLA. (A) Typical recordings of mEPSCs in control conditions and during application of DA. (B) Cumulative distribution of mEPSC amplitude in control and DA conditions (p>0.05, K-S test). (C) Effects of DA on mEPSC amplitude (n=6, p>0.05). (D) Changes of mEPSC frequency during DA application. (E) Cumulative distribution of inter-event intervals of mEPSC before and during DA application (p>0.05, K-S test). (F) Bar graph showing the frequency of mEPSCs in each condition (n=6, p>0.05).

DA did not affect the amplitude and frequency of mEPSCs

The decreases in eEPSCs amplitude could be due to changes in the amount of neurotransmitter released presynaptically and/or due to changes in the postsynaptic response to glutamate. To investigate whether DA (100 μM) application affected eEPSC postsynapticly, we examined the effect of DA application on mEPSC amplitude and frequency. The results showed that DA had no significant effect on the amplitude of mEPSCs (Figure 2A–B, p>0.05). The averaged amplitude of mEPSCs also did not change significantly

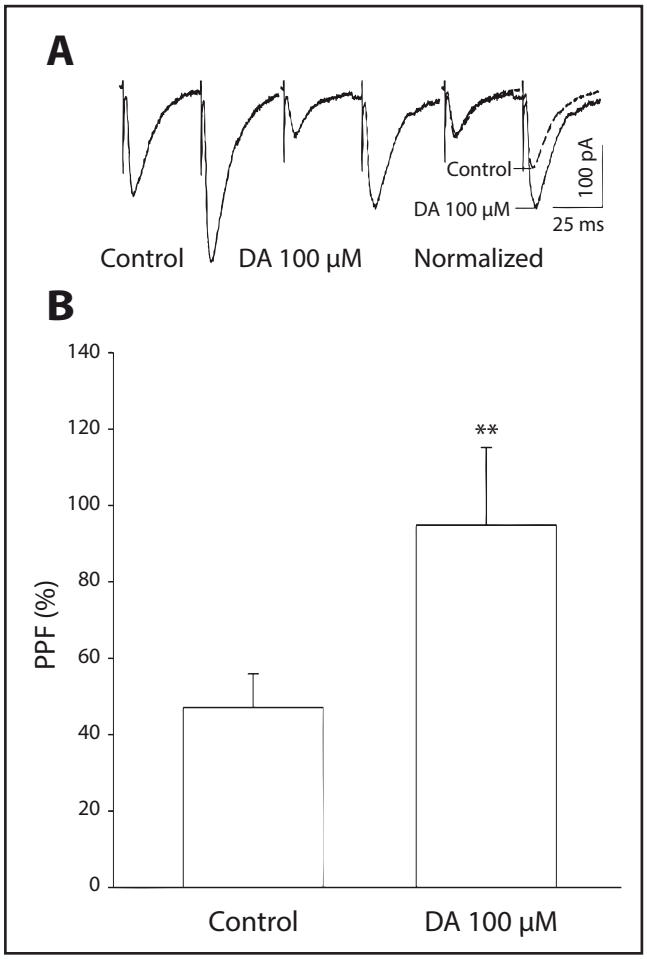

Fig. 3. The effect of DA on PPF in the pyramidal cells of BLA. (A) Representative traces of PPF before and during DA application, as well as the normalized traces for the two conditions. (B) Bar graph showing the effect of DA application on PPF (n=6, $^{**}p<0.01$).

after DA application (Figure 2C, n=6, 14.18±1.00 pA *vs* 14.47±1.03 pA, $p>0.05$). These results is consistent with previous report that DA (50 μM) application had no influence on the non-NMDA receptor mediated currents.

We also found bath application of DA had no significant effect on mEPSC frequencies (Figure 2D–F, 5.12±1.01 Hz *vs* 4.30±1.10 Hz, $p>0.05$).

DA increased PPF

Then we further investigated the effects of DA application on presynaptic terminals by examining the effect of DA on PPF. The PPF was significantly increased from 47.13±8.81% in control condition to 94.86±20.28% at 15 min after DA application (Figure 3A–B, n=6, $p<0.01$). These results suggested that DA may inhibit stimulus-evoked glutamate release.

DA inhibited activity-dependent increase in the frequency of sEPSCs

To further confirm the effect of DA on stimulus-evoked glutamate release, the effect of DA application on KCl and 4-Aminopyridine (4-AP) evoked sEPSCs was studied. First, bath application of KCl and 4-AP, a non-selective voltage-dependent K^+-channel blocker, significantly increased the frequency of sEPSC 15 min after application. The combined application of DA and KCl showed that, DA application significantly decreased the frequency of sEPSC as well as inhibited the effect of KCl (Figure 4A–B, n=6, $p<0.05$). Consistently, DA application also significantly inhibited the effect of 4-AP (200 μM) (Figure 4C–D, n=6, $p<0.05$).

The effect of DA on EPSCs was independent of the activation of D1 and D2-like receptors

To investigate the involvement of dopaminergic receptors in DA mediated effects, we examined the effects of the D1-like antagonist SCH23390 and D2-like antagonist sulpiride in dopamine-dependent inhibition of eEPSCs. The results showed the inhibitory effects of DA on the amplitude of eEPSCs were not affected by bath application of both antagonists. The inhibition of the amplitude of eEPSC by DA application was 54.99±4.78%, while such inhibition after application of SCH23390, sulpiride and combined use of SCH23390 and sulpiride were 59.41±4.38%, 60.55±2.73% and 58.87±9.78%, respectively (Figure 5A–B, n=6, $p>0.05$). The results showed that both D1-like antagonist and D2-like antagonist failed to block dopamine-dependent inhibition.

To further confirm these results, we studied the influence of DA receptor agonists on the amplitude of evoked EPSCs. The normalized eEPSCs amplitudes were 90.59±17.56%, 87.04±8.79% and 92.86±14.12%, respectively, after application of SKF38393 (10 μM), Quinpirole (10 μM) and combined application of those two agonists. There was no significant changes of eEPSC amplitude, as compared with the control group of DA application (101.41±6.88%) (n=6, $p>0.05$).

DISCUSSION

DA is a neuroendocrine transmitter that synthesized by neurons from the substantia nigra, ventral tegmental area and the medial zona interna (Momiyama *et al.* 1996), which has been shown to modulate both excitatory and inhibitory synaptic transmission (Anzalone *et al.* 2012; Cornil & Ball 2008; Diaz *et al.* 2011). In the present study, we studied the effect of DA application on excitatory neurotransmission of the pyramidal cells in the BLA.

We first studied the effect of DA application on evoked EPSCs in the BLA of rat at 14–16 days old. We found that bath application of DA at the concentration of 100 μM significantly inhibited the amplitude of evoked EPSC with the suppression rate of 54.99±4.78%. Such effect can be reversed by ACSF washout. This result is different from the previous work that DA increased excitability of basolateral amygdala neurons in adult rodents (Kroner *et al.* 2005; Pickel *et al.* 2006). We suppose such difference could be due to different

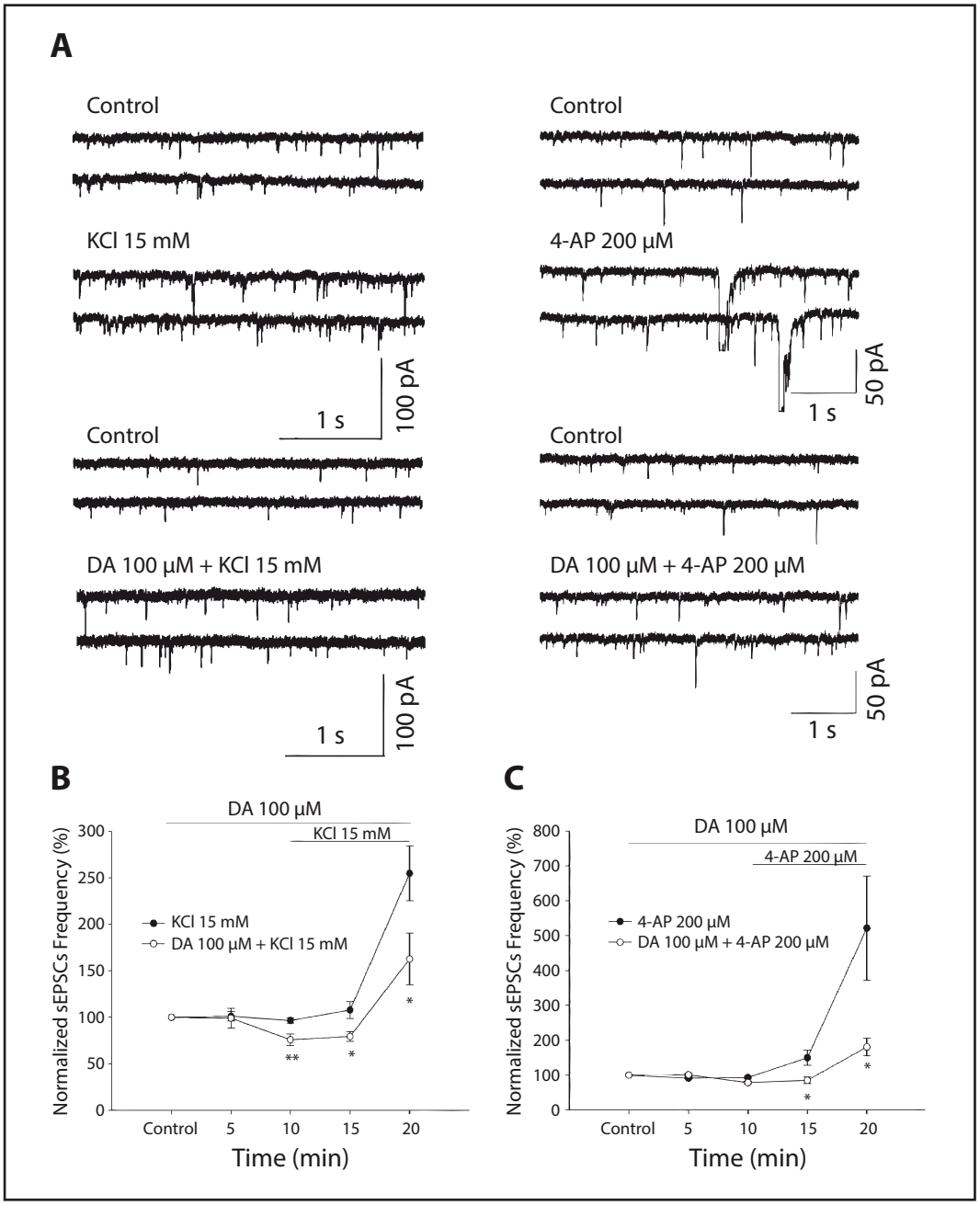

Fig. 4. The effect of DA on activity induced changes in sEPSCs in the pyramidal cells of BLA. (A) Representative recordings of sEPSCs in the absence and presence of KCl and DA. (B) Time course study showing the effect of DA and KCl (15 mM) on sEPSC frequencies. (n=6, * $p<0.05$, compared with KCl group). (C) Representative recordings of sEPSCs in the absence and presence of 4-AP and DA. (D) Time course study showing the effect of DA and 4-AP (200 μM) on sEPSC frequencies. (n=6, * $p<0.05$, compared with 4-AP group).

ages of animals studied, that our work mainly focused on the effect of DA on developing rats.

We also studied the mechanism of DA effect in the BLA. To study the postsynaptic effect of DA, we first examined mEPSCs of pyramidal neurons in the BLA to measure changes in the postsynaptic AMPA receptor-mediated responses. However, we did not observe any difference in both the amplitude and frequency of mEPSCs, indicating the effect of DA on BLA is probably not through postsynaptic mechanism. An alternative hypothesis is that DA affected excitatory neurotransmission via changes in the pre-synaptic neurotransmitter release. Thus we examined PPF to evaluate pre-synaptic release probability. The results showed that PPF was significantly increased from 47.13±8.81% in control condition to 94.86±20.28% at 15 min after DA application. Since changes in PPF mostly due to pre-synaptic alterations (Zucker & Regehr 2002), these

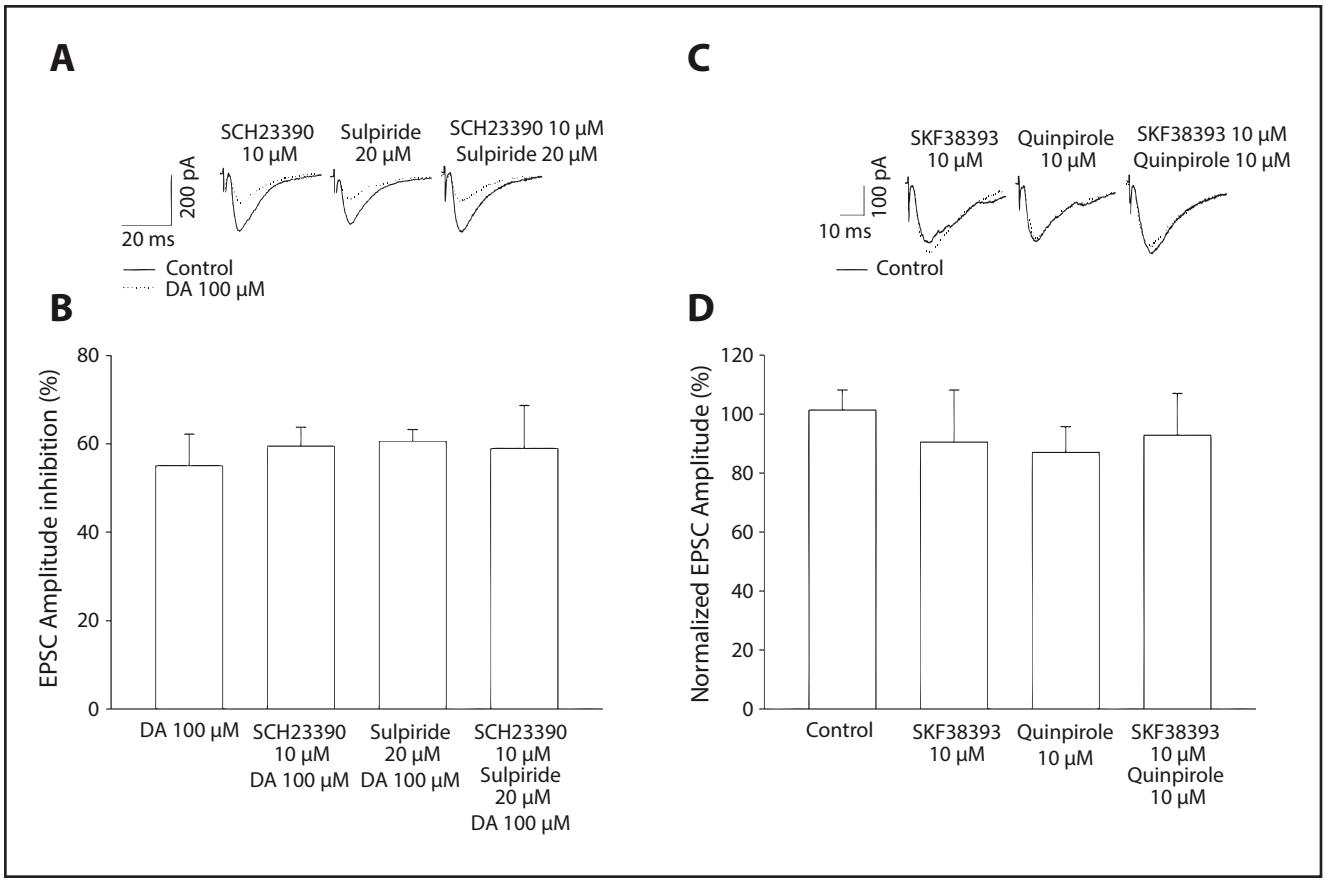

Fig. 5. DA receptors were not involved in DA induced changes on eEPSCs. (A) Representative recordings of eEPSCs in conditions as indicated. (B) Bar graph showing the effect of antagonists for D1 and D2-like receptors (n=6, $p>0.05$). (C) Representative recordings of eEPSCs in conditions as indicated. (D) Bar graph showing the effect of agonists for D1 and D2-like receptors (n=6, $p>0.05$).

results suggested that DA may inhibit stimulus-evoked glutamate release through pre-synaptic mechanism.

We also investigated the effect of DA application on the amplitude and frequency of spontaneous EPSCs to evaluate activity dependent quantal release of excitatory neurotransmitter. We observed that DA application significantly decreased the frequency of sEPSC in the BLA. Moreover, we studied the effect of DA on KCl and 4-AP treated neurons, which are common treatment to increase neural activity. The results showed that DA significantly decreased the activity induced upregulation in both the amplitude and frequency of sEPSCs. Thus, we further demonstrated the inhibitory effect of DA on projection neurons in BLA.

There are two types of dopamine receptors, including D1-like receptor and D2-like receptor, both of which have been reported to inhibit presynaptic glutamate release (Harvey & Lacey 1996; Koga & Momiyama 2000; Shen & Johnson 2012; Yuen *et al.* 2010). To further study the underlying mechanism of DA application on excitatory neurotransmission, we examined the effects of administration of the agonists and the antagonists for both D1 and D2-like receptors on eEPSCs. Our results showed that none of the two DA receptor agonists, including SKF38393 and quinpirole, were capable of mimicking the effect of DA in decreasing eEPSC amplitude. Consistently, neither SCH23390 nor sulpiride, both of which are DA receptor antagonists, abolished the inhibitory effect of DA on pyramidal neurons in the BLA. These results indicated that regulation of excitatory neurotransmission by DA on BLA neurons is not dependent on dopaminergic receptors during development. Further studies on the mechanism through which DA modulates excitatory synaptic transmission in the BLA are needed to examine whether other receptors are required in DA induced inhibition on excitatory neurons in the BLA.

The BLA plays important roles in regulation of emotional behaviors, such as fear responses, pain and anxiety (Sah *et al.* 2003). By studying the effect of DA application on excitatory neurotransmission in the BLA, we found the role of DA in decreasing the eEPSC and sEPSC in pyramidal neurons through a pre-synaptic mechanism during development. These results shed light on our understanding of the regulation of neural excitability by dopamine application and provide insight to study the role of DA on the function of the BLA during development.

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