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Dopamine Modulates Corticostriatal Synaptic Transmission through Both D_1 and D_2 Receptor Subtypes in Rat Brain

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Striatum has important roles in motor control, habitual learning and memory. It receives glutamatergic inputs from neocortex and thalamus, and dopaminergic inputs from substantia nigra. We examined effects of dopamine (DA) on the corticostriatal synaptic transmission using *in vitro* extracellular recording technique in rat brain corticostriatal slices. Synaptic responses were elicited by stimulation of cortical glutamatergic inputs on the corpus callosum and recorded in the dorsal striatum. Corticostriatal population spike (PS) amplitudes were decreased (39.4±7.9%) by the application of 100 μM DA. We applied receptor subtype specific agonists and antagonists and characterized the modulation of corticostriatal synaptic transmission by different DA receptor subtypes. D_2 receptor agonist (quinpirole), antagonist (sulpiride), and D_1 receptor antagonist (SKF 83566), but not D_1 receptor agonist (SKF 38393), induced significantly the reduction of striatal PS. Pretreatment neither with SKF 83566 nor sulpiride significantly affected corticostriatal synaptic inhibition by DA. However, the inhibition of DA was completely blocked by pretreatment with mixed solution of both SKF 83566 and sulpiride. These results suggest that DA inhibits corticostriatal synaptic transmission through both D_1 and D_2 receptors in concert with each other.

Key Words: Dopamine, Population Spike, Striatum, Rat

INTRODUCTION

Dopamine (DA) is an important neurotransmitter in the central nervous system, where it controls a variety of functions including locomotor activity, cognition, emotion, positive reinforcement, food intake, and endocrine regulation (Missale et al, 1998). Dopaminergic neurons are located primarily within the substantia nigra pars compacta (Andersen & Jansen, 1990) and ventral tegmental area, and give rise to dense efferent projections, terminating in the dorsal and ventral striatum, as well as limbic cortex and associated subcortical structures (West et al, 2003) The molecular and pharmacological characterization of DA receptors has been an important issue to understand the normal brain function and the pathophysiology of several neurologic and psychiatric diseases (Sokoloff & Schwartz, 1995). Five DA receptor subtypes have been identified, and they are divided into two families, based on their pharmacological and biochemical characteristics. D_1 and D_5 receptors constitute the D_1-like family of receptors, and these receptors activate G_s proteins, therefore, stimulate adenylyl cyclase. D_2, D_3, and D_4 receptors constitute the D_2-like family of receptors, and these receptors activate Gi proteins, which inhibit adenylyl cyclase.

The neostriatum (caudate and putamen) is involved in the control of movement (Groves, 1983; Graybiel et al, 1994; Mink, 1996), and it appears that certain forms of learning and memory involve changes in neostriatal function (Jog et al, 1999). Glutamatergic inputs from the cortex and thalamus are the major efferents determining the activity of medium spiny neurons (MSNs), which are principal neurons and comprise more than 90% of striatal cells (Kawaguchi, 1997). Dopaminergic inputs from the substantia nigra onto the MSNs modulate these corticostriatal glutamatergic inputs and have important roles in the synaptic transmission and plasticity of striatal cells. Experimental manipulations in rodents or Parkinson's disease in humans showed that striatal DA depletion results in a mark deterioration of the corticostriatal synaptic transmission (Ingham et al, 1998; Meshul & Allen, 2000).

Several electrophysiological studies showed that DA modulates synaptic transmission and plasticity in the striatum (Calabresi et al, 1992; Hsu et al, 1995; Levine et al, 1996; Umemiya & Raymond, 1997; Centonze et al, 2001; Cepeda et al, 2001). However, it is still remains controversial through which mechanisms DA modulates corticostriatal synaptic transmission in the striatum, especially since there exist 5 different subtypes of the receptor. Therefore, using *in vitro* extracellular recording technique with the corticostriatal slice, we examined modulatory mechanism of DA involved in corticostriatal glutamatergic synaptic transmission.

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ABBREVIATIONS: DA, dopamine; PS, population spike; MSN, medium spiny neuron; aCSF, artificial cerebrospinal fluid.

METHODS

Preparation of brain slice

Brain slices were prepared from 15- to 25-day-old Sprague Dawley rats, using previously described technique (Sung et al, 2001). Rats were killed by decapitation, and brains were quickly removed and placed in ice-cold, modified artificial CSF (aCSF) containing (in mM) 194 sucrose, 30 NaCl, 4.5 KCl, 1 $MgCl_2$, 26 $NaHCO_3$, 1.2 NaH_2PO_4, 10 D-glucose and pH adjusted to 7.4 by bubbling with 95% O_2/5% CO_2. The brain which contains the cerebral cortex and striatum was coronally sectioned (300~400 μm thick) with manual vibratome (Campden Instrument, Lough-borough, UK). Brain slices were transferred to aCSF containing (in mM) 124 NaCl, 4.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 26 $NaHCO_3$, 1.2 NaH_2PO_4, 10 D-glucose and pH adjusted to 7.4 by bubbling with 95% O_2/5% CO_2, and allowed to equilibrate for at least 1 hour at room temperature. One hemislice containing the cortex and striatum was then transferred to a recording chamber. The slices were submerged and constantly superfused (at a flow rate of 2~3 ml/min) with aCSF constantly bubbled with 95% O_2/5% CO_2 through a peristaltic pump (Miniplus 2, Gilson, France). The temperature of the bath solution was kept at 31±1°C.

Recording of extracellular field potential

All recordings were performed in the dorsolateral striatum. The population spikes (PS) were evoked by stimulation of excitatory afferents from cerebral cortex. Electrical stimuli were delivered through a bipolar, Teflon-coated tungsten electrode placed in the white matter dorsal to the striatum. Synaptically driven PS were recorded with a glass micropipette (<1 MΩ tip resistance) filled with 0.9% saline, which was placed at a striatum 1~2 mm ventral to the stimulating electrode. The position of the recording electrode was optimized by recording responses to low frequency stimulation (0.02~0.2 ms, 0.5~1.5 mA at 0.1 Hz), and the electrode was set at the depth where the maximal PS amplitude was observed. Stimulus intensity was then adjusted to evoke a PS with amplitude approximately half of the maximum by stimulus isolator (A360, WPI, Sarasota, USA). Once a PS of half-maximal amplitude triggered by 0.05 Hz stimulus was stably maintained for 10 to 15 min, drugs were then delivered. Field potentials were amplified 1000× using a differential AC amplifier (Model1700, A-M systems, Seattle, WA), and low-pass filtered at 5 kHz. Amplified signals were digitized using a CIO-DAS08/JR-AO interface (Measurement Computing Corporation, Middleboro, MA, USA) and stored on a computer using LTP230d program (Anderson & Collingridge, 2001). Drugs from stock solutions were dissolved in aCSF to their final concentrations and delivered to the recording chamber. Drug-containing solutions were allowed to equilibrate in the recording chamber for at least 3~4 min.

Data analysis

All averaged data were presented as means±SE. Amplitudes of the first 30 PS before application of drug were averaged and defined as baseline responses, and drug responses were compared with this value. The statistical significance of changes in synaptic responses relative to baseline response amplitude was determined using a Student's paired *t*-test. The statistical criterion for significance was $P<0.05$.

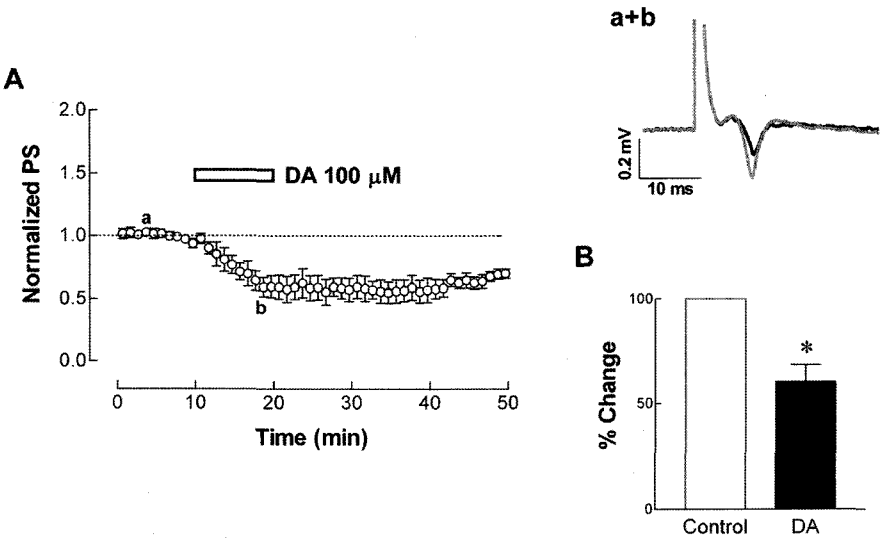

Fig. 1. DA depresses the amplitude of population spike (PS) on corticostriatal synaptic transmission. After stable baseline was recorded under normal aCSF for 10 min, DA was added for 10 min. (A) Normalized plotting averaged PS graph showing that bath application of 100 μM DA decreased the PS amplitude. The bar shows the period of DA application. (a), (b) Representative trace to show baseline PS under normal aCSF and PS under DA treatment. (B) Bar graph showing averaged PS data. The baseline is PS which was recorded for 10 min under normal aCSF. Representative superimposed PS traces for each experiment are presented below the graph plotted. *$P<0.01$, compared with baseline.

RESULTS

Inhibition of glutamatergic synaptic transmission by DA

The field potential evoked by stimulation of corpus callosum consists of two negative spikes. It has recently been reported that the first spike is a pre-synaptic fiber volley and the second spike is a synaptically induced population spike (Choi et al, 2003). Therefore, we used amplitudes of the second spike to analyze population spikes that were synaptically induced by the activation of corticostriatal

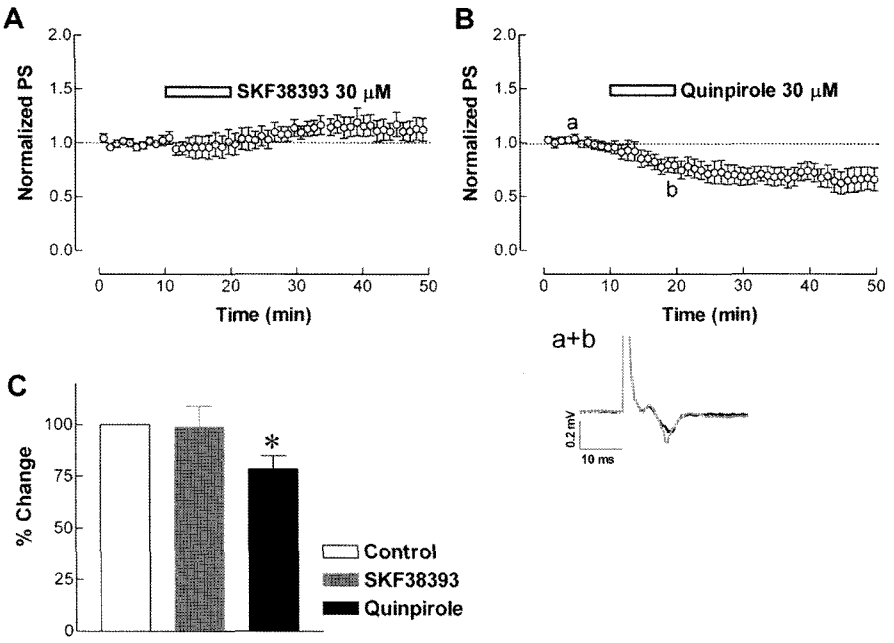

Fig. 2. Effect of synaptic transmission mediated by DA receptor subtype specific agonists. (A) Plotted averaged data showing that PS was not changed by application of SKF 38393, D_1 receptor agonist. (B) Plotted data showing that application of quinpirole, D_2 receptor agonist, decreased the PS amplitude. (C) Bar graph shows averaged PS data. $^*P<0.01$, compared with baseline.

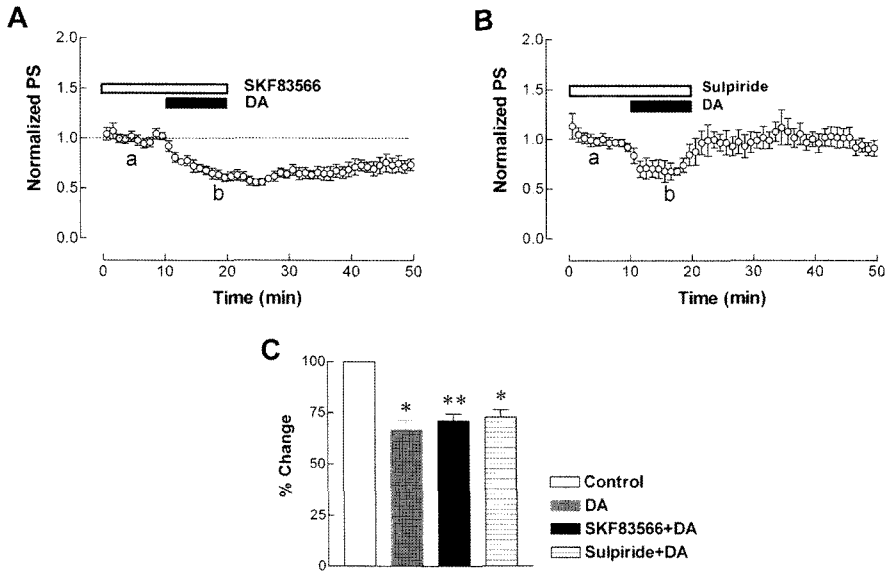

Fig. 3. DA still decreased the corticostriatal PS in the presence of either D_1 or D_2 receptor antagonist. (A), (B) Plotted PS data showing that PS was depressed by co-application of 100 μM DA with SKF 83566, D_1 receptor antagonist, or 10 μM sulpiride, D_2 receptor antagonist. (C) Bar graph showing inhibition of PS by co-application with DA and DA receptor antagonists. $^*P<0.01$, $^{**}P<0.001$, compared with baseline.

afferents. As shown in Fig. 1, superfusion of DA containing aCSF into the recording chamber for 10 min decreased the amplitude of PS: At 100 μM concentration, the average PS amplitude was decreased by 39.4±7.9% (n=5, $P<0.01$) of the baseline. PS amplitude rapidly decreased when treated with DA, and the maximal inhibition was observed after 10 min of DA treatment. The inhibitory effect continued for over 30 min after washout of DA.

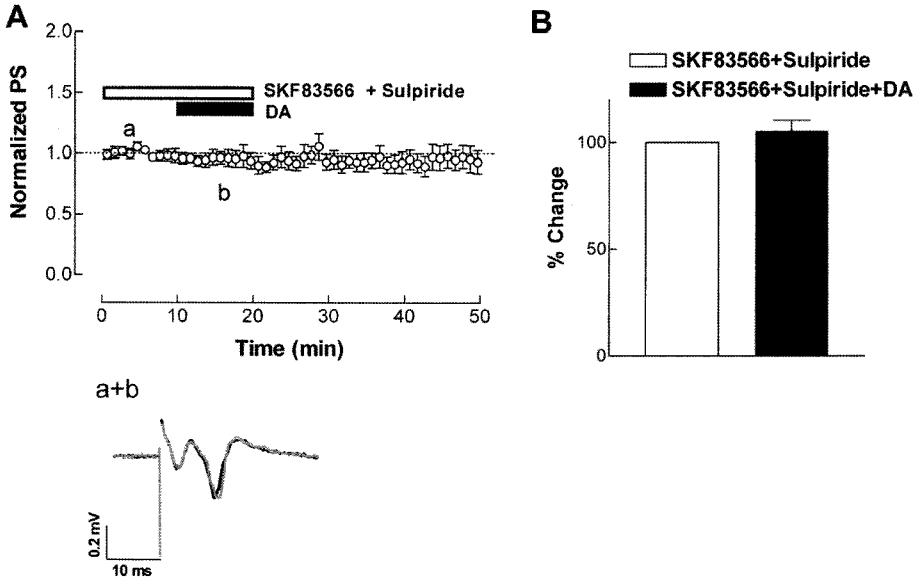

Fig. 4. Inhibitory effect of DA is mediated by both D_1 and D_2 receptors. (A) Plotted averaged PS data showing that depression of DA was completely blocked by co-application of DA with 10 μM each of both SKF 83566 and sulpiride. (B) Bar graph shows averaged data. The difference was not significant.

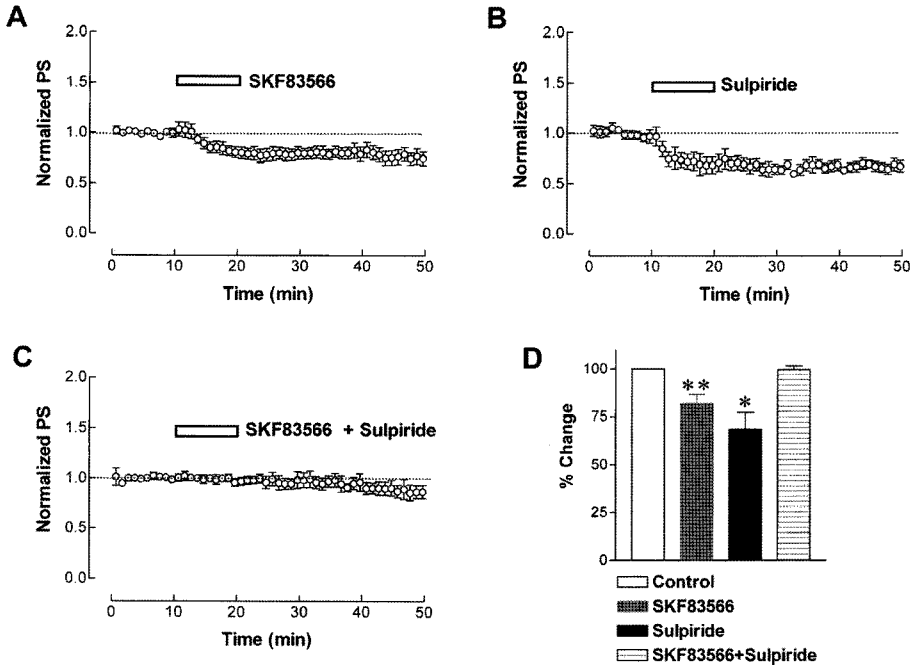

Fig. 5. Effect of a mixture of DA receptor antagonists on the corticostriatal synaptic transmission. (A), (B) Plotted data showing that striatal PS was depressed by application of SKF 83566 or sulpiride. (C) Plotted data showing that the PS amplitude was not changed by application of mixed solution of both. (D) Bar graph shows averaged PS data. *$P<0.05$, **$P<0.01$, compared with baseline.

Response to D_1 and D_2 receptor subtype specific agonists

Next, we examined whether agonists for different subtypes of DA receptor affected differently the corticostriatal synaptic transmission. As shown in Fig. 2A, application of 10 μM SKF 38393, a D_1 receptor agonist, resulted in no significant change ($1.1 \pm 10.2\%$, n=7, P=0.9145) in the amplitude of PS on corticostriatal synaptic transmission. However, quinpirole (30 μM), a D_2 receptor agonist, decreased the PS amplitude by 22.6 ± 6.7 (n=5, $P<0.05$) from the baseline (Fig. 2B).

Block of both D_1 and D_2 receptor were necessary to prevent the effects of DA

To examine which subtype of DA receptor was involved in these inhibitory effects of DA, slices were pre-incubated for 10 min with the D_1 and D_2 receptor antagonists, respectively. In the presence of 10 μM SKF 83566, D_1 receptor antagonist, the amplitude of PS was still decreased by $29.0 \pm 3.3\%$ (n=5, $P<0.001$) of the baseline by the application of 100 μM DA (Fig. 3A). After pre-treatment with 10 μM sulpiride, D_2 receptor antagonist, the PS amplitude was also decreased by $27.3 \pm 3.7\%$ (n=4, $P<0.01$). In this case, the field response was recovered reversibly toward the baseline after wash out of DA (Fig. 3B). Next, we examined the effect of DA after blockade of both D_1 and D_2 receptors. Pretreatment with a mixed solution of both SKF 83566 and sulpiride significantly blocked the decrease of PS amplitude by the application of 100 μM DA (5.4 ± 5.2, n=5, P=0.3567, Fig. 4). These data indicate that D_1 and D_2 receptors were simultaneously, not alone, blocked to inhibit DA action on the corticostriatal synaptic transmission. In support of the above data, Fig. 5 shows the effects of each DA receptor antagonists alone as a mixed solution of both on the corticostriatal synaptic transmission. Field response was decreased by $18.2 \pm 4.7\%$ or $31.4 \pm 8.5\%$ by bath application of SKF 83566 (n=8, $P<0.01$) or sulpiride 10 μM (n=5, $P<0.05$), respectively. However, there was no significant change of PS amplitude by the mixed solution (-0.4 ± 1.9, n=5, P=0.8486).

DISCUSSION

In the present study, we examined the inhibitory action of DA on corticostriatal synaptic transmission. Our results indicate that DA acts as an inhibitory modulator on the corticostriatal synaptic transmission, and these regulatory actions were mediated by both D_1 and D_2 subtypes of dopamine receptor.

Using the various electrophysiological experiments, a number of previous studies have reported the actions of DA in the striatum (Calabresi et al, 1992; Cepeda et al, 1993; Hsu et al, 1995; Hu & White, 1997; Umemiya & Raymond, 1997; Tang et al, 2001). In the striatum as well as in other brain areas, DA receptors showed opposite responses, depending on the receptor subtypes activated by agonist. D_1 receptor activation enhances synaptic responses elicited by glutamate receptors, which are mediated by *N*-methyl-*D*-aspartate (NMDA) receptors, whereas D_2 receptor activation reduces synaptic responses, which are mediated by activation of non-NMDA receptors (Cepeda et al, 1993; Cepeda & Levine, 1998). The enhancing effects of D_1 receptor activation appear to involve post-synaptic action (Flores et al, 1999), whereas the attenuating effects mediated by D_2 receptors may involve both pre- and post-synaptic actions (Levine et al, 1996). Our present data showed that, DA, D_1 receptor antagonist (SKF 83566), D_2 receptor agonist (quinpirole), and D_2 receptor antagonist (sulpiride) declined the field responses on corticostriatal synaptic transmission (Fig. 2B, 5A). The result is basically in agreement with a previous work which showed suppression of synaptic response by D_1 receptor antagonist and D_2 receptor agonist (Hsu et al, 1995). We also confirmed that the application of SKF 38393, D_1 receptor agonist, had no significant effect on corticostriatal synaptic transmission (Fig. 2A). In recent studies, the effect of SKF 38393 on corticostriatal evoked-EPSCs has been well documented (Umemiya & Raymond, 1997), and the EC_{50} of SKF 38393 for increasing neostriatal intracellular cAMP levels was on the order of 0.4 μM (Andersen et al, 1990; Andersen & Jansen, 1990), These facts suggest that higher concentrations of SKF38393 not only possibly saturated the effects mediated by D_1-type receptors, but also activates D_2-type DA receptors (Andersen & Jansen, 1990; Seeman & Van Tol, 1994). Thus, we speculate that our results with 30 μM SKF 38393 may reflect the possibility of co-activation of D_1- and D_2-type receptors during the perfusion of drug.

There are many views about the role of D_1 receptor in various brain regions, and It still remains debated whether corticostriatal synaptic transmission is potentiated by D_1 receptor activation. Some studies have shown that D_1 receptor causes a depression of excitatory synaptic transmission in striatum (Surmeier et al, 1992; Pacheco-Cano et al, 1996). On the other hand, however, others have documented that the inhibitory effect of D_1 receptor takes place only in ventral striatum, but not dorsal striatum (Nicola & Malenka, 1998). As seen in Fig. 2B, we observed that bath application of sulpiride, D_2 receptor antagonist, decreased the PS amplitude on corticostriatal synaptic transmission. Therefore, it appears highly likely that the depression of corticostriatal synaptic transmission by sulpiride could be mediated by D_1 receptor or another D_2 subtype receptor. However, it needs a further study to elucidate these possibilities.

Although the role of pre-synaptic D_2 receptor is more predominant than post-synaptic D_1 receptor in corticostriatal synapse, early studies documented that the function of DA receptors in the glutamatergic synaptic transmission was dependent on the concentration of DA used (Hsu et al, 1995). They suggested that DA at a low concentration (≤ 0.1 μM) affects only pre-synaptic glutamate release mechanisms via the activation of pre-synaptic D_2 receptor. However, at a higher (>0.1 μM) concentration, it can also modulate post-synaptic responses to excitatory amino acids through the activation of both post-synaptic D_1 and D_2 receptors and depress the excitatory synaptic transmission. Our results showed that, neither the pretreatment of SKF 83566 nor sulpiride before DA application prevented the inhibitory action of DA (Fig. 3A, B). These results support the idea that both D_1 and D_2 receptor subtypes may be involved in the regulation of corticostriatal synaptic transmission, therefore, blockade of D_1 or D_2 receptor alone could not be enough to prevent DA effects. These possibilities were also supported by our results which showed no change of striatal PS by co-application of both SKF 83566 and sulpiride. However, we could not exclude the possible limitation of PS recording technique, because *in vitro* extracel-

lular recording technique measures electrical signal which is evoked in field where neuronal groups exists, but not single neuron. To gain an information about action site where DA is activated between pre- and post-synaptic receptor on corticostriatal synapse, further study is needed by using whole-cell patch clamp recording in single neuron and by measuring paired-pulse ratio (PPR) and spontaneous EPSC, which detects the change of neurotransmitter release when electrical stimulation is not applied.

In conclusion, therefore, we could suggest that DA induces the depression on corticostriatal synaptic transmission, and that these inhibition of DA is mainly be due to co-activation of both D_1 and D_2 receptors.

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