Dopamine Presynaptically Depresses Fast Inhibitory Synaptic Transmission via D4 Receptor-Protein Kinase A Pathway in the Rat Dorsolateral Septal Nucleus

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Dopamine presynaptically depresses fast inhibitory synaptic transmission via D4 receptor-protein kinase A pathway in the rat dorsolateral septal nucleus. J Neurophysiol 96: 591–601, 2006. First published April 26, 2006; doi:10.1152/jn.00966.2005. The lateral septal nucleus receives a diffuse dopaminergic input originating from the ventral tegmental area of the brain stem. We examined whether dopamine (DA) modulates synaptic transmission in the slice preparation of the rat dorsolateral septal nucleus (DLSN). Bath application (10–15 min) of DA (30 μM) markedly depressed the amplitude of fast and slow inhibitory postsynaptic potentials (IPSPs) in DLSN neurons, while it produced only a minor depression of the amplitude of excitatory postsynaptic potentials (EPSPs) obtained in the presence of bicuculline. DA (30 μM) depressed the monosynaptic fast IPSP to ~50% of control, but did not depress the inward current (I_GABA) induced by exogenous γ-aminobutyric acid (GABA). DA decreased the frequency of miniature fast IPSPs (m-IPSPs) without significantly changing their amplitude. PD 168077, a selective D4 receptor agonist, depressed the fast and slow IPSPs but not the EPSP and decreased the frequency of m-IPSPs. Both DA and PD 168077 increased the paired-pulse ratio of the monosynaptic fast IPSP. The inhibitory effect of DA on the fast IPSP was significantly attenuated by L-741,742, an antagonist at D4 receptors, but not by SCH 23390 and sulpiride, a D1-like and a D2-like receptor antagonist, respectively. N-ethylmaleimide, a blocker of pertussis toxin (PTX)-sensitive G protein (G_o), attenuated the DA-induced depression of the fast IPSP. N-[2-((p-bromocinnamyl) amino)methyl]-5-isouquinoline sulfonamide, a protein kinase A (PKA) inhibitor, attenuated the DA-induced depression of the fast IPSP. These results suggest that DA inhibits spontaneous and evoked release of GABA via the D4 receptor-G Protein-PKA system in DLSN neurons.

INTRODUCTION

The lateral septum is an integral part of the limbic system that controls a variety of behaviors related to higher cognitive functions (e.g., learning and memory), emotions, fear, aggression, stress, and autonomic regulation (Lisciotto et al. 1990; Paxinos 1975; Simon et al. 1986; Thomas et al. 1991). Massive glutamatergic projections from the hippocampal CA1 and CA3 pyramidal layers form excitatory synapses on neurons in the lateral septum (Joëls and Urban 1984; Meibach and Siegel 1977; Swanson and Cowan 1979). GABAergic principal neurons in the lateral septum send efferents to various target neurons in the CNS, such as prefrontal cortex, hypothalamic nuclei, and lower brain stem nuclei (Blume et al. 1982; Meibach and Siegel 1977; Swanson and Cowan 1979). A diffuse dopamine (DA) input from neurons in the A10 (ventral tegmental area: VTA) and A14-15 cell groups forms dense plexuses around cell body and proximal segments of the somatotopically neurons in the lateral septum (Adams and Moghaddam 2000; Jakab and Leranth 1995; Moore and Bloom 1978). DA-containing nerve terminals in the lateral septum may play an important role in regulating emotional behaviors. Lesions of the lateral septum enhanced aggressive behaviors elicited by electrical stimulation of the ventromedial hypothalamic nucleus (Maeda 1978; Maeda and Maki 1987). Administration of DA precursors or agonists attenuates the rage behavior produced by chronic septal lesions (Gage and Olton 1976; Marotta et al. 1977) or the facilitation of hypothalamic rage by chronic septal lesions (Maeda and Maki 1987).

Functional studies using brain slice preparations have shown that DA has a complex effect on neuronal excitability and synaptic transmission in the CNS. DA produces both hyperpolarizing (Lacey et al. 1988; Mercuri et al. 1992; Uchida et al. 2000; Uchimura et al. 1986) and depolarizing responses (Bernardi et al. 1982; Shi et al. 1997; Yang et al. 1991; Zhu et al. 2002) in central neurons. DA presynaptically enhances the frequency and amplitude of miniature inhibitory postsynaptic currents (IPSCs; GABAergic) in pyramidal neurons in upper cortical layers (Miyazaki and Lacey 1998; Zhou and Hablitz 1999). DA receptors are classified into two receptor families, namely the D1- and D2-like receptor families (Jaber et al. 1996; Missale et al. 1998; Seeman and Van Tol 1994). The D1 family, which positively couples to adenylate cyclase, consists of the D1 and D5 receptor subtypes, whereas the D2 family, which negatively couples to adenylate cyclase, consists of the D2-D4 receptors (Bergson et al. 1995). In the rat, DA presynaptically inhibits IPSCs in supraoptic neurons via D4 receptors (Azzad et al. 2003) and in prefrontal cortex neurons via D1 receptors (Gonzalez-Islas and Hablitz 2001). DA inhibits postsynaptic GABA_A receptor channel function by depressing protein kinase A (PKA)-protein phosphatase system via D4 receptors in prefrontal cortex neurons (Wang et al. 2002). Autoradiographic or immunocytochemical imaging studies demonstrated that both D1- and D2-like receptors are expressed in lateral septal neurons (Charuchinda et al. 1987; Ciliax et al. 2000; Khan et al. 1998; Mansour et al. 1990; Primus et al. 1997). Stimulation of the ventral tegmental

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dopaminergic projection produces an excitation in lateral septum neurons by acting at D2-like receptors (Assaf and Miller 1977). In contrast, iontophoretic application of DA reduces spontaneous activity in lateral septal neurons in vivo (Joëls and Urban 1985). A recent study showed that DA produced a membrane hyperpolarization and blocked spontaneous action potentials in septal neurons (Asaumi et al. 2005). Investigating the effect of DA on synaptic transmission should contribute to understanding the functional role of DA in the dorsolateral septal nucleus (DLSN) because few studies have been made to examine the effects of DA on the inhibitory synaptic transmission in this area. The purpose of the present study was to investigate, in detail, the effects of DA on the inhibitory synaptic transmission in DLSN using conventional electrophysiological techniques. Preliminary findings of this work have appeared in abstract form (Asaumi et al. 2004, 2005).

METHODS

The experimental protocols were approved by the Animal Research Committee of the Kurume University School of Medicine. All efforts were made to minimize suffering and the number of animals used. Transverse slices of rat brain containing the septal nuclei were obtained in a manner described previously (Hasuo et al. 2002). Briefly, male Wistar rats (4–6 wk; 120–220 g) were killed by decapitation, and septal slices with 400 μm thickness were prepared with a vibroslice (Campden Instruments). These slices were incubated in artificial cerebral spinal fluid (ACSF) containing (in mM) 117 NaCl, 4.7 KCl, 1.2 MgCl₂, 2.5 CaCl₂, 1.2 NaH₂PO₄, 11 glucose, and 25 NaHCO₃ oxygenated with 95% O₂-5% CO₂ and kept at room temperature for ≈1 h before experimentation. The slices were superfused with warm (31–33°C), oxygenated, ACSF at 2 ml/min. Conventional intracellular microelectrodes filled with KCl were fused with warm (31–33°C), oxygenated, ACSF at 2 ml/min. Conventional intracellular microelectrodes filled with KCl were fused with warm (31–33°C), oxygenated, ACSF at 2 ml/min. Conventional intracellular microelectrodes filled with KCl were fused with warm (31–33°C), oxygenated, ACSF at 2 ml/min.

Drugs were purchased from the following sources: DA, bicuculline, GABA, DNQX, AP5, forskolin, tetrodotoxin (TTX), and N-ethylmaleimide (NEM) were purchased from Sigma-Aldrich (St Louis, MO). SKF 38393, quinpirole, PD 168077, SCH 23390, sulpiride, and CGP 55845 were from Tocris Cookson, UK. N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinoline sulfonamide (H-89) was purchased from Seikagaku, Sulpiride and SCH 23390 were dissolved in ethanol. PD 168077, L-741,742, H-89, DNQX, CGP 55845, and forskolin were dissolved in dimethyl sulfoxide (DMSO) and added to the ACSF. The final concentration of DMSO (0.1%) and ethanol (0.1%) had no direct effect on DLSN neurons. DA and other drugs were added to the ACSF (adjusted pH or osmolality) and applied by superfusion. To reduce oxidation of DA, L-ascorbic acid (3 μM in final concentration) was added to the ACSF.

Statistical analysis was made by the paired t-test, the two-tailed Student’s t-test or one-way ANOVA followed by post hoc test. To detect significant differences between two means, a paired t-test or Student’s t-test was used. For comparison of multiple groups, a one-way ANOVA was performed followed by post hoc test. The amplitude and frequency distributions of miniature IPSPs were compared using the nonparametric Kolmogorov-Smirnov test. Significance was assessed at P < 0.05. All data are expressed as means ± SE.

RESULTS

Effects of DA on synaptic transmission in the DLSN

DLSN neurons had resting membrane potential of –61.8 ± 0.4 mV (n = 89) and input resistance of 103 ± 3.0 MΩ (n = 89). DA (1–10 μM) had no effect on the membrane potential and the input membrane resistance of DLSN neurons (n = 34). DA (30 μM) applied to the bath for 2 min produced a small hyperpolarization in 31 neurons (3.2 ± 0.3 mV), a small depolarization in 8 neurons (2.7 ± 0.5 mV), and no change in membrane potential in 10 neurons. The pooled data showed that the hyperpolarization induced by DA (30 μM) was associated with a significant decrease in the input membrane resistance (88.7 ± 1.8% of control, n = 31, P < 0.05). On the other hand, no significant change in the input membrane potential of the fast IPSP was rarely observed during experiments, and data obtained from the cells that have tendency of show “run-down” of the IPSPs within 20 min were discarded because these cells were not healthy. The change in amplitude of the fast IPSP could be caused by the shift of membrane potential. We controlled the membrane potential carefully to minimize the gradual membrane potential change by injecting DC currents to the neurons. All reported measurements of postsynaptic potentials were the average of six consecutive responses.

γ-Aminobutyric acid (GABA; 2 mM) was directly applied to neurons by pressure pulses (140 kPa for 6 ms) through a broken-tip micropipette to evaluate the GABA receptor sensitivity at the postsynaptic membrane. The monosynaptic fast IPSP induced by a paired-pulse stimulation was recorded in the presence of DNQX (20 μM), AP5 (50 μM), and CGP 55845 (4 μM). Two synaptic responses were evoked by a couple of stimuli given at 200-ms intervals. Paired-pulse ratio (PPR) was expressed as the ratio of the amplitude of second synaptic response to the first synaptic response. When miniature fast IPSPs (m-IPSPs) were recorded from DLSN neurons, recording microelectrodes were filled with CsCl (2 M) to block K⁺ channels in DLSN neurons. m-IPSPs were recorded as a depolarizing response at the membrane potential of –65 mV. Continuous recordings of 3-min epochs were obtained with Clampex software. Analysis of the properties of m-IPSPs was made with mini-analysis software (Synaptosoft). Individual m-IPSPs were accepted for analysis if they had amplitude of >0.2 mV, were monophasic, and decayed to base line in an approximately exponential fashion.
resistance was observed in other two subgroups of neurons (depolarization group: 98.2 ± 1.4% of control, n = 8, P > 0.1 and no-change group: 96.1 ± 3.3% of control, n = 10, P > 0.1, respectively). A single stimulation of the fimbria/fornix pathway that contains the main axons of hippocampal (CA1 and CA3) neurons evoked an EPSP followed by fast and slow IPSPs (Fig. 1A). Application of DA (30 μM) to the ACSF for 15 min reversibly depressed the amplitude of fast and slow IPSPs, while it increased the amplitude of the EPSP to 125–300% (n = 8) of control (Fig. 1B). Figure 1B shows the time course of the effects of DA (30 μM) on the EPSP and IPSPs in a DLSN neuron. The facilitation of the EPSP and depression of fast and slow IPSP appeared within minutes after the beginning of application of DA and lasted for 20–30 min after removal of DA (Fig. 1B). The fast IPSP partially overlaps with both the EPSP and slow IPSP in DLSN neurons (Gallagher et al. 1995).

To examine the effects of DA on the EPSP and slow IPSP, the fast IPSP was blocked by bicuculline (15 μM), a GABA_A receptor antagonist (Fig. 2A). The EPSP had mean amplitude of 5.1 ± 0.3 mV (n = 40) and relaxed with a half-decay time of 28.4 ± 1.5 ms (n = 40) in the presence of bicuculline (15 μM). The amplitude and half-decay time of the slow IPSP were 7.0 ± 0.4 mV (n = 40) and 275 ± 8.8 ms (n = 40), respectively. Figure 2A shows an example of the DA effect on the EPSP and slow IPSP in a DLSN neuron superfused with ACSF containing bicuculline (15 μM). Application of DA (30 μM) for 12 min depressed the EPSP and slow IPSP to ~80 and 50% of control, respectively. Washout of DA for 20 min from the external solution almost completely reversed the observed effects. The DA-induced depression of the slow IPSP was observed irrespective of the type of the membrane-potential-change induced by DA. Figure 2B shows pooled data for the effect of DA (1–100 μM) on the EPSP. The amplitude of the EPSP was 94 ± 3% (n = 35) and 78.6 ± 6.0% (n = 7) of control in the presence of DA at concentration of 30 and 100 μM, respectively. The concentration-response relationship for DA showed that at concentration of 3, 10, 30, and 100 μM, DA depressed the amplitude of the slow IPSP to 91.3 ± 2.8% (n = 7), 73.8 ± 3.1% (n = 24), 52.9 ± 3.0% (n = 35), and 37.2 ± 7.8% (n = 7) of control, respectively (Fig. 2B).

Presynaptic inhibition of the fast IPSP in monosynaptic GABAergic pathway

It has been shown that the fast and slow IPSPs were disynaptically mediated by GABA because GABAergic projection neurons are innervated by glutamatergic excitatory nerve inputs via the fimbria/fornix pathway (Gallagher et al. 1995; Hasuo et al. 1992; Jakab and Leranth 1995). To examine the direct effect of DA on GABAergic transmission, the monosynaptic fast IPSP was recorded from DLSN neurons, where the EPSP and slow IPSPs were blocked by DNQX (20...
The fast IPSP was blocked by bicuculline (15 μM/11005 (30 μM) reached maximum 5 min after beginning of application of DA course of the effect of DA on the fast IPSP. The depression of the fast IPSP were 5.8 ± 0.8% near the recorded neuron. The amplitude and half-decay time of DA (30 μM) for 15 min depressed the fast IPSP to ~50% of control (Fig. 3A). Figure 3Ba shows the time course of the effect of DA on the fast IPSP. The depression reached maximum 5 min after beginning of application of DA (30 μM), was maintained as long as DA (30 μM) was present in the external solution, and recovered within 20 min after re-application of recovery solution. Figure 3Bb shows a concentration-dependent depression of the fast IPSP induced by DA (1–1,000 μM). The minimal and near maximal concentrations of DA to depress the fast IPSP were 3 and 300 μM, respectively. These results suggest that DA directly inhibits GABAergic neurotransmission in the DLSN.

The effect of DA on the sensitivity of postsynaptic GABA<sub>A</sub> receptors was examined by discontinuous single-electrode voltage clamp (dSEVC) to minimize the effect of conductance change on GABA responses at postsynaptic membrane of DLSN in neurons. DLSN neurons were superfused with an external solution containing CGP 55845 (4 μM) to block GABA<sub>B</sub> receptors that mediate the slow IPSP. Cs<sup>+</sup> was injected into DLSN neurons by diffusion from an intracellular recording microelectrode filled with 2 M CsCl to block K<sup>+</sup> channels in DLSN neurons. GABA (2 mM), applied directly to DLSN neurons by a pressure pulse (140 kPa for 6 ms) through a broken-tip micropipette, produced an inward current (I<sub>GABA</sub>) with amplitude of 287 ± 23 pA (n = 8) at −80 mV (Fig. 3Ca). The I<sub>GABA</sub> was blocked by bicuculline (15 μM; not shown). When DA (100 μM) was applied to the neurons, no obvious change in the amplitude of I<sub>GABA</sub> occurred in this neuron (Fig. 3Cd). The pooled data showed that the amplitudes of I<sub>GABA</sub> was 103 ± 6.8% (n = 8) of control in the presence of DA (100 μM). There was no statistical significant difference between the I<sub>GABA</sub> amplitude obtained in the absence and the presence of 100 μM DA (P > 0.5). These results suggest that DA does not change the sensitivity of postsynaptic GABA<sub>A</sub> receptors on DLSN neurons.

**DA receptor subtype that contributes to the effect of DA on GABAergic transmission**

The subtype of DA receptors mediating the depression of the fast IPSPs was determined by using selective agonists and antagonists. Figure 4Aa shows an example of the effects of PD 168077, a selective D4 receptor agonist (Glase et al. 1997), on the EPSP and slow IPSP in a DLSN neuron. Application of PD 168077 (30 μM) for 15 min produced a depression (~50% of control) of the slow IPSP. However, PD 168077 did not alter the amplitude of EPSPs in the same cell. Pooled data showed that the amplitude of EPSPs was 91.0 ± 3.9% (n = 6) of control during the application of PD 168077 (30 μM), suggesting that PD 168077 produced no significant depression of the EPSP (P > 0.05). The amplitude of slow IPSPs was depressed to 44.6 ± 1.5% (n = 6) of control by application of 30 μM PD 168077 (P < 0.01; Fig. 4Ab). The magnitude of the depression of slow IPSPs induced by PD 168077 (30 μM) was similar to that induced by DA (30 μM) in DLSN neurons (Fig. 2Bb). The effects of DA receptor agonists on the monosynaptic fast IPSP were examined in DLSN neurons treated with DNQX (20 μM), APS (50 μM), and CGP 55845 (4 μM; Fig. 4B). Figure 4Ba shows an example of the effect of PD 168077 (10 μM) on the fast IPSP in a DLSN neuron. Bath-application of PD 168077 (10 μM) for 15 min depressed the fast IPSP to ~70% of control. Figure 4Bb shows that PD 168077 depressed the amplitude of monosynaptic fast IPSP in a dose-dependent manner. The magnitude of depression of the monosynaptic fast IPSP induced by PD 168077 (300 μM) was similar to that induced by DA (1000 μM). Bath-application of SKF 38393 (10 μM), the D1-like receptor agonist, for 10–15 min did not
DOPAMINE D4 RECEPTOR-MEDIATED INHIBITION OF IPSPs

Effects of DA and PD 168077 on PPR of the fast IPSP in DLSN neurons

The monosynaptic fast IPSP induced by a paired-pulse stimulation was recorded in the presence of DNQX (20 μM), APS (40 μM), and CGP 55845 (4 μM). If the release probability of GABA is depressed, the PPR would increase (Kerrick and Zhuo 2002). We measured the peak amplitude of the fast IPSPs evoked by a pair of pulses with an inter-stimulus interval of 200 ms and calculate the amplitude ratio of the second to the first IPSP in each pair. Figure 5 shows an example of these experiments. In the absence of DA, the average amplitude ratio of the second to the first IPSP was 0.81, indicating paired-pulse depression. When DA (30 μM) was applied to the external solution, both the first and second IPSPs in the pair decreased their amplitudes. However, the PPR of the fast IPSP was increased to 0.88 in this neuron (Fig. 5Aa). Figure 5Ab shows pooled data for the facilitation of the PPR induced by DA. Application of DA (30 μM) for 10–15 min significantly increased the PPR of the fast IPSP from 0.79 ± 0.04 to 0.89 ± 0.04 (n = 5, P < 0.05). DA (30 μM) increased the PPR to 114 ± 1% (n = 5) of control. Figure 5B shows the effect of PD 168077 (10 μM) on the PPR of the fast IPSP. PD 168077 (10 μM) increased the PPR from 0.82 to 0.94. Figure 5Bb shows the pooled data for facilitation of the PPR induced by PD 168077. PD 168077 (10 μM) significantly increased the PPR of the fast IPSP from 0.79 ± 0.04 to 0.86 ± 0.03 (n = 4, P < 0.05). PD 168077 (10 μM) increased the PPR to 110 ± 2% (n = 4) of control. These results suggest that DA depresses the evoked release of GABA via activation of presynaptic D4 receptors in DLSN neurons.
Figure 6b shows the effect of DA (100 μM) on the frequency of m-fIPSPs. DA shifted the cumulative distribution of the mean inter-event interval of the m-fIPSP to the right, indicating a longer inter-event interval. Pooled data showed that DA (100 μM) decreased the frequency of m-fIPSPs from 1.80 ± 0.33 Hz (n = 4) to 0.57 ± 0.16 Hz (n = 4, P < 0.05). At a concentration of 30 μM, DA depressed the frequency of m-fIPSPs from 1.23 ± 0.13 Hz (n = 4) to 0.70 ± 0.04 Hz (n = 4) (P < 0.05) without changing their amplitude (data not shown). The effect of PD 168077 (30 μM) on the m-fIPSP was also examined in DLSN neurons. Bath application of PD 168077 (30 μM) produced no change in the cumulative amplitude distribution of the m-fIPSP (Fig. 6Ca). In contrast, PD 168077 (30 μM) caused a shift in the inter-event interval distribution toward longer intervals (Fig. 6Cb). The mean frequencies of m-fIPSPs were 1.41 ± 0.26 Hz (n = 3) and 0.40 ± 0.03 Hz (n = 3) in the absence and the presence of PD 168077 (30 μM), respectively (P < 0.05). Furthermore, L-741,742 (10 μM), a D4 receptor antagonist, attenuated the DA (10–30 μM)-induced depression of m-IPSP-frequency (data not shown). These data suggest that DA decreases spontaneous release of GABA via D4 receptors in DLSN neurons.

M-fIPSPs were recorded from DLSN neurons perfused with ACSF containing tetrodotoxin (TTX) (1 μM), DNQX (20 μM), AP5 (40 μM), and CGP 55845 (4 μM). Cs⁺ and Cl⁻ were injected into DLSN neurons by diffusion from an intracellular microelectrode filled with 2M CsCl. The m-fIPSPs were recorded from the DLSN neurons as depolarizing responses under these conditions (Fig. 6Aa). The m-fIPSP was completely blocked by bicuculline (15 μM; data not shown). Averaged amplitude of the m-fIPSPs recorded within 3 min were compared between the data obtained before and during application of DA for 9 min. The mean amplitude of m-fIPSP was 1.09 ± 0.04 mV (n = 4) in the absence of DA. DA (100 μM) produced no visible change in the membrane potential (−65 ± 0.6 mV, n = 4) in Cs⁺-treated neurons. In the same cells, DA (100 μM) reduced the m-fIPSPs frequency (Fig. 6Ab). Figure 6Ba shows that the cumulative distribution of m-fIPSPs amplitudes was not altered by DA (100 μM); the mean amplitude of m-fIPSPs was 1.05 ± 0.03 mV (n = 4) in the presence of DA (100 μM). The mean amplitude of m-fIPSPs in the presence of DA was not different that obtained in the absence of DA (P > 0.5).

Effects of DA and PD 168077 on m-fIPSP in DLSN neurons

M-fIPSPs were recorded from DLSN neurons perfused with ACSF containing TTX (1 μM), DNQX (20 μM), AP5 (40 μM), and CGP 55845 (4 μM). Cs⁺ and Cl⁻ were injected into DLSN neurons by diffusion from an intracellular microelectrode filled with 2M CsCl. The m-fIPSPs were recorded from the DLSN neurons as depolarizing responses under these conditions (Fig. 6Aa). The m-fIPSP was completely blocked by bicuculline (15 μM; data not shown). Averaged amplitude of the m-fIPSPs recorded within 3 min were compared between the data obtained before and during application of DA for 9 min. The mean amplitude of m-fIPSP was 1.09 ± 0.04 mV (n = 4) in the absence of DA. DA (100 μM) produced no visible change in the membrane potential (−65 ± 0.6 mV, n = 4) in Cs⁺-treated neurons. In the same cells, DA (100 μM) reduced the m-fIPSPs frequency (Fig. 6Ab). Figure 6Ba shows that the cumulative distribution of m-fIPSPs amplitudes was not altered by DA (100 μM); the mean amplitude of m-fIPSPs was 1.05 ± 0.03 mV (n = 4) in the presence of DA (100 μM). The mean amplitude of m-fIPSPs in the presence of DA was not different that obtained in the absence of DA (P > 0.5).
Intracellular signal transduction in the DA-induced inhibition of the fast IPSP

The properties of the G protein that mediates the DA-induced inhibition of the fast IPSP was examined in DLSN neurons. Figure 7A shows examples of the effect of DA on the fast IPSP in the absence and the presence of NEM (100 μM), a membrane-permeable inhibitor of pertussis toxin (PTX)-sensitive G proteins (Nakajima et al. 1990; Shapiro et al. 1994). Before applying NEM, we confirmed that DA (30 μM) produced a typical depression of the fast IPSP amplitude (53.1 ± 4.4% of control, n = 6, P < 0.05) in a DLSN neuron. After the recovery of fast IPSPs, application of NEM (100 μM) for 30–60 min (Matsuoka et al. 2004) produced no significant change in the amplitude of fast IPSPs in the same DLSN neuron (101 ± 2.5% of control, n = 6, P > 0.1). Pooled data showed that DA (30 μM) depressed the fast IPSP to 83.4 ± 2.4% of control (n = 6, P < 0.05) after treatment of NEM (100 μM). The magnitude of the depression was significantly smaller than that in the absence of NEM (P < 0.05; Fig. 7B). The result that NEM significantly attenuates the DA-induced depression of the fast IPSP suggests that a PTX-sensitive G protein (Glic) contributes to the DA-induced depression of the fast IPSP in DLSN neurons.

It is known that the D4 receptor is negatively coupled to the adenylate cyclase-PKA pathway (Missale et al. 1998; Wang et al. 2002). If the D4 receptor-mediated inhibition of the IPSPs was due to a decrease in the concentration of intracellular cyclic AMP (cAMP), forskolin (10 μM), an activator of adenylate cyclase, would enhance the fast IPSP. Bath application of forskolin (10 μM) produced no obvious change in the resting membrane potential (−63.9 ± 1.6 mV, n = 7) for control and −63.4 ± 1.2 mV, n = 7 for forskolin, P > 0.1) but increased the amplitude of fast IPSPs to 151 ± 11% (n = 8, P < 0.05) of control (Fig. 8A). The forskolin-induced facilitation of the fast IPSP lasted for 10–20 min and recovered 40 min after the removal of forskolin from the external solution. We examined the contribution of cAMP-dependent protein kinase on the DA-induced depression of the fast IPSP in DLSN neurons. Figure 8B shows the direct effect of H-89 (10 μM), a PKA inhibitor, on the fast IPSP. H-89 (10 μM) has been reported to block PKA-mediated actions in prefrontal cortex neurons (Wang et al. 2002; Young and Yang 2004). H-89 (10 μM) almost completely blocked the forskolin (10 μM)-induced facilitation on fast IPSP to 95.9 ± 3.7% of control (n = 3, data not shown). Application of H-89 (10 μM) itself produced a slight decrease in the amplitude of fast IPSPs (88.1 ± 4.5% of control, n = 4). However, these data are not significantly different (P > 0.05). Figure 8C shows the effects of DA on the fast IPSP in the absence and presence of H-89. Application of DA (30 μM) produced a typical depression of the fast IPSP.
IPSP (47.2 ± 2.2% of control, n = 10) in the absence of H-89. After DA (30 μM) was removed from the external solution, neurons were then treated with an external solution containing H-89 (10 μM) for 1–2 h. Addition of DA (30 μM) to the external solution containing H-89 (10 μM) depressed the amplitude of fast IPSPs to 71.1 ± 2.1% of control (n = 4). As shown in Fig. 8C, the DA-induced depression of the fast IPSP was significantly attenuated in the presence of 10 μM H-89 (P < 0.01). Furthermore, we examined the effect of H-89 (100 μM), at relatively high concentration (Marabese et al. 2005), on the DA-induced depression of the fast IPSP. The effect of DA (30 μM) on the fast IPSP was examined in DLSN neurons incubated in the H-89-containing ACSF solution for 1–2 h. As a result, H-89 (100 μM) almost completely blocked the DA-induced depression of the fast IPSP (the amplitude of the fast IPSP in the presence of DA was 94.7 ± 3.7% of control, n = 5). These results suggest that the adenylate cyclase-PKA system is involved in the DA-induced inhibition of the IPSP in DLSN neurons.

DISCUSSION

Inhibition of GABAergic transmission by DA

The present study clearly showed that application of DA (10–30 μM) for 10–15 min depressed the amplitude of fast and slow IPSPs in DLSN neurons. DA (30 μM) applied to the bath for 2 min produced a small hyperpolarization in 31 neurons (mean 2.7 mV), a small depolarization in 8 neurons (mean 3.2 mV), and no change in membrane potential in 10 neurons. The DA (30 μM) induced depression of the IPSPs was observed irrespective of the type of the membrane potential change induced by DA. Even in the neurons which showed the DA (30 μM)-induced hyperpolarization, the change in input membrane resistance was apparently less than the ratio of the depression of the IPSP. The influence due to the change in membrane potential was minimal, since the membrane potential change was nullified by injecting DC current during application of DA. Furthermore, DA (10 μM) produced a significant depression of the IPSPs without altering the membrane potential or the input membrane resistance. These data suggested that the effect of DA in depressing GABAergic synaptic transmission is essentially independent of the change of membrane potential and input resistance.

The present study also showed that DA enhanced the amplitude of EPSPs. The facilitation of EPSPs induced by DA is probably due to the depression of the fast IPSP because the EPSP was not enhanced by the application of DA (30 μM) to neurons, when the fast IPSP was blocked by bicuculline (15 μM), a GABA_A receptor antagonist. Furthermore, DA (30 μM) directly depressed the fast IPSP in monosynaptic GABAergic pathway. Such an inhibition of GABAergic transmission may consequently enhance the EPSP under physiological condition because the fast IPSP has been known to overlap with the EPSP in DLSN neurons (Gallagher et al. 1995). DA did not affect the I_GABA produced by direct application of GABA to the neurons. DA increased the PPR of the monosynaptic fast IPSP. DA did not affect the amplitude of m-fIPSPs, whereas it depressed the frequency of the m-fIPSPs in the presence of TTX. These results suggest that DA (10–30 μM) inhibits the IPSP by reducing evoked and spontaneous release of GABA in DLSN neurons. Previously, electrophysiological and morphological studies have shown that stimulation of the fimbria appears to evoke both feedforward and feedback IPSPs in DLSN (Gallagher et al. 1995). Although there is no direct evidence to support the existence of any classical interneuron in the lateral septum, previous data have indicated the presence of “functional” GABAergic local circuit within the DLSN (Phelan et al. 1989). DLSN neurons receive inhibitory fibers from both neighboring DLSN neurons and inhibitory medial septum neurons (Leranth et al. 1992). Because principle neurons are GABAergic in the lateral septum (Jakab and Leranth 1995), it is suggested that DA mainly inhibits the feedforward IPSPs in inhibitory fibers received from neighboring DLSN neurons. However, the possibility of contribution of the feedback IPSPs cannot be ruled out.

Previous studies have reported that DA has multiple and opposite effects on inhibitory synaptic transmission in central neurons. For example, DA presynaptically enhanced the frequency and amplitude of mIPSCs in pyramidal neurons in upper cortical layers (Miyazaki and Lacey 1998; Zhou and Hablitz 1999). In contrast, DA presynaptically inhibited GABAergic synaptic transmission in rat supraoptic neurons (Azdad et al. 2003) and in prefrontal cortex (Gonzalez-Islas and Hablitz 2001). DA postsynaptically modulated GABAergic responses via D4 receptors and D5 receptors (Liu et al. 2000; Wang et al. 2002). The effects of DA in the lateral septum are consistent with those in supraoptic hypothalamic neurons. Previous studies have shown that DA directly enhances the excitatory postsynaptic current (EPSC) in pyramidal neurons of rat prefrontal cortex (Gonzalez-Islas and Hablitz 2003) or inhibits glutamatergic transmission in the rat supraoptic nucleus neurons (Price and Pittman 2001). Recently, we have observed that the EPSP was enhanced by a prolonged application of DA, at a relatively low concentration (1 μM), in DLSN neurons (unpublished observation). However, further studies are needed to clarify whether DA can directly modulate the EPSP in the lateral septum.

D4 receptors mediate the DA-induced depression of the IPSPs

DA receptors are classified into two receptor families, namely the D1- and D2-like receptor families (Jaber et al. 1996; Missale et al. 1998; Seeman and Van Tol 1994). The D1 family further consists of the D1 and D5 receptor subtypes, whereas the D2 family consists of the D2–D4 receptors (Bergson et al. 1995). The present study determined the DA receptor subtype that contributes to the DA-induced presynaptic inhibition of IPSPs in DLSN neurons. This is based on the observation that PD 168077, a selective D4 receptor agonist, mimicked the effect of DA in depressing the fast and slow IPSPs, but it did not alter the EPSP amplitude. Neither quinpirole, a D2-like receptor agonist, nor SKF 38393, a D1-like receptor agonist, produced depression of the IPSP. The DA-induced inhibition of the IPSP was significantly attenuated by L-741,742, a selective D4 receptor antagonist, but not by sulpiride, a D2/D3 receptor antagonist, or by SCH 23390, an antagonist at D1/D5 receptors. Although L-741,742 (50 μM) did not completely antagonize the effects of DA on the fast IPSP, PD 168077 (100–300 μM) strongly depressed the amplitude of the fast IPSP. PD 168077 increased the PPR of the
monosynaptic fast IPSP. PD 168077 also depressed the frequency of the m-fIPSPs without changing their amplitudes in the presence of TTX. These results suggest that D4 receptors are responsible for the presynaptic inhibition of GABAergic transmission to DLSN neurons.

**Signal transduction in the DA-induced inhibition of the fast IPSP**

The present study showed that NEM significantly attenuated the DA-induced depression of the fast IPSP, suggesting that a PTX-sensitive G protein, probably $G_{i/o}$ type, may be involved in the D4 receptor-mediated inhibition of the IPSPs in the DLSN. The application of forskolin to DLSN neurons produced a consistent enhancement of the amplitude of fast IPSPs in DLSN neurons. Furthermore, application of H-89, a selective PKA inhibitor itself, for 1–2 h produced a small depression of the fast IPSP in some neurons. The forskolin (10 μM)-induced facilitation of the monosynaptic fast IPSP was prevented by H-89 (10 μM). The DA-induced inhibition of the fast IPSP was attenuated by H-89 (10 μM) and was almost blocked by H-89 (100 μM). These results suggest that a D4 receptor-cAMP-PKA system is involved in the DA-induced depression of the IPSP in DLSN neurons. Such a PKA-dependent mechanism for the DA-induced inhibition of IPSPs has been reported in supraoptic nucleus and prefrontal cortex neurons (Azdad et al. 2003; Gonzalez-Islas and Hablitz 2001).

**Functional implications of DA effects on the DLSN neurons**

The facilitation of the EPSP induced by inhibition of the fast IPSP may increase the gain of the input-output neuronal response in the physiological solution. The lateral septum sends GABAergic inhibitory efferents to target neurons in the prefrontal cortex, hypothalamic areas as well as lower brain stem nuclei (Blume et al. 1982; Meibach and Siegel 1977; Swanson and Cowan 1979). Therefore disinhibition of neuronal signals in the DLSN yields a strong inhibition of the activity of these target neurons. Clinical and experimental studies have reported that DA is implicated in the pathogenesis of a number of neurological and psychiatric disorders, including cognitive dysfunction, emotional disorders, and schizophrenia (Egan and Weinberger 1997; Yang et al. 1999). Many researches using imaging techniques have suggested that the release of DA is elevated in a certain group of patients with schizophrenia (Abi-Dargham et al. 1998; Breier et al. 1997; Laruelle 2000). Magnetic resonance imaging (MRI) reveals that schizophrenic patients have a larger cavum septum pellucidum than normal subjects (Sheehan et al. 2004). Primus et al. (1997) have suggested that D4 receptors are densely expressed in the lateral septal region next to entorhinal cortex in rat. D4 receptor-knockout mice have an increased level of DA biosynthesis and degradation indicating that D4 receptor has a role as an inhibitory autoreceptors (Rubinstein et al. 1997). Based on our present results, the activation of D4 receptors in DLSN neurons may affect the activity of neurons in not only the DLSN, but also the VTA, because projection neurons (mostly GABAergic) in the DLSN innervate VTA neurons (Sheehan et al. 2004). Furthermore, the lateral septum has been reported to regulate the firing rates of ventral tegmental area (Maeda and Mogenson 1981). If dysfunction of D4 receptors occurs in DLSN neurons, subsequent disinhibition of VTA neurons might enhance the release of DA in the CNS.

Lesions of the lateral septum enhanced aggressiveness elicited by electrical stimulation of the ventromedial hypothalamic nucleus (Gray and McNaughton 1983; Maeda 1978; Maeda and Maki 1987). Administration of the DA and agonists, such as l-3,4-dihydroxyphenylalanine (L-DOPA), apomorphine, and amphetamine, abolished or suppressed the septal lesion-induced enhancement of hyperirritability or hyperreactivity (Gage and Olton 1976; Marotta et al. 1977). Considering some part of the lateral septum remained intact in these experiments, DA agonists-induced reduction of the aggressiveness might be produced by increasing the inhibitory effect of GABAergic projection from the remaining neurons in lateral septum to hypothalamic nucleus (LeVere and LeVere 1982; Maeda and Maki 1987). The results in the present study together with previous reports suggest that the DA-induced enhancement of the activity of GABAergic projection neurons in the lateral septum may result in a strong inhibition of hypothalamic aggressive behaviors.

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