Dopamine Inhibition of Evoked IPSCs in Rat Prefrontal Cortex

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Gonzalez-Islas, Carlos and John J. Hablitz. Dopamine inhibition of evoked IPSCs in rat prefrontal cortex. J Neurophysiol 86: 2911–2918, 2001. Rat prefrontal cortex (PFC) receives substantial dopamine (DA) input. This DA innervation appears critical for modulation of PFC cognitive functions. Clinical and experimental studies have also implicated DA in the pathogenesis of a number of neurological and psychiatric disorders including epilepsy and schizophrenia. However, the actions of DA at the cellular level are incompletely understood. Both inhibitory interneurons and pyramidal cells are targets of DA and may express different DA receptor types. Our recent findings suggest that DA can directly excite cortical interneurons and increase the frequency of spontaneous inhibitory postsynaptic currents (IPSCs). The present study was undertaken to determine the effect of specific DA receptor agonists on evoked (e) IPSCs. Visually identified pyramidal neurons were studied using whole cell voltage-clamp techniques. Bath application of DA 30 μM reduced IPSC amplitude to 80 ± 4% (mean ± SE) of control without any significant change in IPSC kinetics or passive membrane properties. The D1-like DA receptor agonist SKF 38393 reduced IPSC amplitude to 71.5 ± 8%, whereas the D2-like specific agonist quinpirole has no effect on amplitude (94.5 ± 5%). The D1-like receptor antagonist SCH 23390 prevented DA inhibition of IPSC amplitude (98.2 ± 4%), whereas IPSCs were still reduced in amplitude (79.7 ± 4%) by DA in the presence of the D2-like receptor antagonist sulpiride. DA increased significantly paired-pulse inhibition, whereas responses to puff applied GABA were unaffected. Addition of the PKA inhibitor H-8 blocked the effect of DA on IPSCs. These results suggest that DA can decrease IPSCs in layer II–III PFC neocortical pyramidal cells by activating presynaptic D1-like receptors.

INTRODUCTION

Prefrontal cortex (PFC) consists of a group of cortical areas in the most anterior portion of the frontal lobe. These areas have been associated with high-level processes needed for the integration of temporal and spatial factors that govern voluntary, goal-directed behavior (Miller 1999). In rats, the PFC has been defined as the part of cortex that receives inputs from the mediodorsal nucleus of the thalamus and from the dopaminergic cell groups localized in the ventral tegmental area (VTA) (Leonard 1969; Thierry et al. 1986). There is strong evidence that this dopaminergic innervation of PFC is critical for the modulation of cognitive function in rats (Simon et al. 1980; Zahrt et al. 1997), primates (Goldman-Rakic 1995; Sawaguchi and Goldman-Rakic 1994), and humans (Barchas et al. 1994; Iversen 1995; Okubo et al. 1997). Clinical and experimental studies have implicated dopamine (DA) in the pathogenesis of a number of neurological and psychiatric disorders, including epilepsy (Starr 1996) and schizophrenia (Andreason 1996; Egan and Weinberger 1997; Grace et al. 1997; Jaskiw and Weinberger 1992; Yang et al. 1999).

Immunohistochemical studies in rat and primate cortex have shown that DA terminals, together with glutamatergic axon terminals, form so called synaptic triads on dendritic spines of pyramidal neurons. DA activation can thus gate excitatory synaptic inputs to pyramidal neurons (Goldman-Rakic 1992; Williams and Goldman-Rakic 1995). In addition, DA axon terminals form symmetric contacts with dendritic spines and shafts of pyramidal neurons as well as with the dendrites of inhibitory interneurons (Benes et al. 1993; Sesack et al. 1995; Verney et al. 1990; Williams and Goldman-Rakic 1993). Pyramidal neurons and interneurons may express different or multiple subtypes of DA receptors (Mrzljak et al. 1996; Vincent et al. 1993, 1995) providing a means for differential DA modulation of cortical neurons. Therefore dopaminergic activation may shift, in a complex way, the balance between excitation and inhibition in neuronal circuits, providing a wide range of possibilities for dopaminergic modulation in PFC.

Although multiple DA receptors have been cloned (Missale et al. 1998), DA receptors are functional characterized into two pharmacologically identifiable families. The D1-like receptor family (composed of D1 and D5 receptor subtypes) is preferentially coupled to subtype specific members of the G_i-like protein family that stimulate the activity of adenylyl cyclase (AC) and protein kinase A (PKA)-dependent pathways. The D2-like receptor family (comprised of D2–D4 subtype receptors) couples to subtype specific members of the G_0o-like protein family and inhibit the same AC-PKA pathway. This variety in receptor and effector mechanisms, coupled with regional heterogeneity in expression and synapse organization, complicates understanding of the DA system in PFC.

A detailed characterization of the cellular mechanisms underlying DA effects on PCF neurons is still emerging. In vivo extracellular recordings have shown that spontaneous firing of rat prefrontal neurons is depressed by DA application (Sesack and Bunney 1989; Thierry et al. 1992) and by VTA stimulation (Ferron et al. 1984). Both N-methyl-D-aspartate (NMDA) and AMPA receptor-mediated excitatory postsynaptic potentials (EPSPs) in layer V pyramidal cells are decreased by DA via a D1 receptor (Law-Tho et al. 1994). DA has also been shown to enhance the induction of long-term depression in layer V cells (Law-Tho et al. 1995; Otani et al. 1998). Although DA innervation of layer V is significantly higher than in layers II/III, upper cortical layers receive a substantial DA input (Emson...
and Koob 1978; Vincent et al. 1993). Our previous work suggests that DA increases the excitability of GABAergic interneurons and enhances the frequency and amplitude of spontaneous inhibitory postsynaptic currents (IPSCs) in interneurons and pyramidal neurons in upper cortical layers (Zhou and Hablitz 1999).

Differential effects of neuromodulators on evoked, spontaneous, and miniature synaptic currents have been reported in cerebellar stellate cells (Kondo and Marty 1998) and hippocampal neurons (Pitler and Alger 1992; Scanziani et al. 1992, 1993). In the present study, we have used whole cell voltage-clamp recordings to examine the effects of DA on evoked inhibitory postsynaptic currents (IPSCs) in rat layer II–III pyramidal cells. We found that DA, apparently acting through presynaptic D1-like DA receptors, can decrease IPSC amplitude in neocortical pyramidal neurons. Some of these results have been published in abstract form (Gonzalez-Islas and Hablitz 1999).

METHODS

Brain slices were prepared from 76 male Sprague-Dawley rats 15–22 days old. Animals were housed and handled according to approved guidelines. The procedures for preparing slices have been described previously (Zhou and Hablitz 1996). After decapitation under ketamine anesthesia, the brain was removed quickly. Coronal brain slices (~300 μm) were cut from the anterior portion of the brain on a Vibratome. The anterior cingulate cortex and the shoulder (Fr2) region of the frontal cortex (Paxinos and Watson 1986) were examined. These two areas make up a large portion of the prefrontal cortex (Kolb 1990).

The slices were incubated for ≥1 h at room temperature before recording in artificial cerebrospinal fluid (ACSF) containing (in mM) 125 NaCl, 3.5 KCl, 2.5 CaCl2, 1.3 MgCl2, 26 NaHCO3, and 10 D-glucose. ACSF was continuously bubbled with a mixture of 95% O2-5% CO2 to maintain a pH of ~7.4. After incubation, the slices were transferred to a recording chamber with a volume of 1 ml and continuously perfused (3 ml/min) with oxygenated ACSF. A Zeiss Axioskop FS microscope equipped with Nomarski optics, a ×40 water-immersion lens and infrared illumination was used to view neurons in the slices.

Whole cell patch-clamp recording techniques were used. Tight seals (>2 Ω before breaking into whole cell mode) were obtained without cleaning the cell. Patch electrodes had an open tip resistance of ~3 MΩ. Series resistance during recording varied from 10 to 20 MΩ among different neurons and was not compensated. Recordings were terminated whenever significant (>20%) increases in series resistance occurred. The intracellular solution for recording synaptic currents contained (in mM) 135 CsCl, 10 HEPES, 2 Mg-ATP, 0.2 Na-GTP, and 0.5 EGTA. pH and osmolarity were adjusted to 7.3 and 300 mOsm, respectively. Possible liquid junction potentials (calculated to be approximately ~4 mV) were not subtracted from the data presented in the following text. All voltage-clamp recordings were made at a holding potential ~70 mV. K-glutamate-based intracellular solutions were used to record resting membrane and action potentials in current-clamp mode. The composition of this solution was (in mM) 10 KCl, 110 K-glutamate, 10 HEPES, 2 Mg-ATP, 0.2 Na-GTP, and 0.5 EGTA; pH was adjusted to 7.3 and osmolarity to 300 mOsm. Synaptic responses were evoked with a bipolar electrode placed 100–150 μm below and slightly lateral to the recording pipette. Stimuli were constant-current square-wave pulses 50–100 μA as in amplitude (50–100-μs duration). Stimulation frequency was 0.05 Hz. In paired-pulse stimulation experiments, an interpulse interval of 50 ms was used. Whole cell currents were acquired using a Warner PC 505A amplifier (Warner Instruments) controlled by Clampex 7.0 software (Axon Instruments). Responses were filtered on-line at 5 kHz, digitized at 10 kHz, and analyzed using Clampfit 7.0 software (Axon Instruments).

IPSC amplitudes were measured as the difference between baseline and peak. In paired-pulse experiments, an exponential curve was fitted to the decay phase of the first (control) IPSC. The difference between this curve and the peak of the second IPSC was used to determine the second (test) IPSC amplitude. A paired-pulse ratio was calculated by dividing the test IPSC amplitude by the control IPSC amplitude. For experiments with pressure application, GABA (10–100 μM) was pressure applied to the soma of the recorded neuron under direct visual guidance. Pipettes for pressure applications were fabricated in the same manner as patch electrodes described in the preceding text. GABA was applied in a solution consisting of 125 NaCl, 3.5 KCl, 20 HEPES, and 10 glucose (pH 7.3 with NaOH). Pressure applications were controlled using a Picospitzer II (General Valve). Five- to 10-ms pulses were delivered at 3–9 psi. These settings were kept constant during recording.

All recordings were done at room temperature (~22°C). Data are expressed as means ± SE. Statistical analysis of synaptic current amplitudes before, during, and after dopaminergic agents was carried out using two-tailed Student’s t-test with Statmost software (Data-Most). P < 0.05 was considered significant. The synaptic currents shown in the figures represent the average of 10 consecutive responses.

GABAergic receptor-mediated IPSCs were evoked in the presence of 20 μM D(-)-2-amino-5-phosphovaleric acid (D-APV) and 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) to block ionotropic glutamate receptors. DA was used to mimic the endogenous agonist for DA receptors. Selective agonists and antagonists for D1- and D2-like receptors [D1-like agonist SKF 38393 hydrobromide and antagonist SCH 23390 hydrochloride; D2-like agonist quinpirole and antagonist (RS)-(-)-sulpiride] were purchased from Tocris. For paired-pulse experiments, 10 μM SCH 50911 (Tocris) was bath applied to block GABAA receptors. The PKA inhibitor H-8 was purchased from Calbiochem. Sodium metabisulfite (Na2S2O5, 50 μM), used as an antioxidant to protect DA agents from oxidation (Sutor and ten Bruggencate 1990), and bicuculline methiodide were obtained from Sigma. All the drugs were bath applied. Drugs were prepared as concentrated stock solutions, frozen, and dissolved in ACSF prior to each experiment in the final concentration indicated.

RESULTS

DA and passive properties

Pyramidal neurons were identified by their depth below the pia, pyramidal shape and presence of a prominent apical dendrite. Under direct visualization, layer II/III pyramidal neurons in anterior cingulate cortex and the Fr2 region of frontal cortex were recorded in current clamp. No difference between neurons from the two brain areas was found, and data were pooled. Under current-clamp conditions, PFC pyramidal neurons did not fire spontaneously. When injected with depolarizing current pulses, cells fired long-duration (base duration, ~5 ms) spikes that showed adaptation during long pulses. These spiking properties are characteristic of regular spiking pyramidal neurons, as previously reported (McCormick et al. 1985; Zhou and Hablitz 1996, 1999). In the presence of 10 μM bicuculline, 20 μM D-APV, and 10 μM CNQX to prevent the influence of neurotransmitter-dependent conductances, layer II/III PFC pyramidal neurons had a resting membrane potential of ~57.2 ± 1 mV (n = 10) when using a K-glutamate-based intracellular solution. Input resistance, measured by fitting a curve to the linear part of I-V relationship obtained with hyperpolarizing
current pulses, was 327.4 ± 43 Ω (n = 10). A 200-ms depolarizing current pulse evoked 5.6 ± 0.2 (n = 10) action potentials. In these 10 cells, bath application of 30 μM DA did not change significantly the membrane potential (−54.6 ± 4 mV, P > 0.1) or input resistance (353.7 ± 75 MΩ, P > 0.5). Additionally, there was no change in the number of spikes evoked in the presence of DA when the same depolarizing current pulse was applied (5.4 ± 0.2, P > 0.5). These results suggest that under the present experimental conditions, changes in neuronal firing and passive properties are unlikely to underlie DA effects on inhibitory synaptic transmission.

**DA reduces evoked IPSCs in layer II–III PFC pyramidal neurons**

Under direct visualization, 36 layer II–III pyramidal neurons from PFC were studied under voltage-clamp conditions. To test the effect of DA on isolated evoked IPSCs, excitatory postsynaptic currents (EPSCs) were blocked with 20 μM D-APV and 10 μM CNQX. After obtaining a stable whole cell recording, IPSCs were evoked at 0.05 Hz. Representative responses are shown in Fig. 1. After a mean latency of 3.2 ± 0.5 ms (n = 36), an inward current was observed. Under control conditions, IPSCs had a half rise time of 2.3 ± 0.3 ms and decayed exponentially with a time constant (τ) of 26.3 ± 5 ms (n = 36; Fig. 1A). By varying the holding potential, these currents were found to reverse at −6.6 ± 2 mV (n = 36), near the expected chloride equilibrium potential (E_cl = 1.2 mV in our recording conditions). In addition, bath application of 10 μM bicuculline completely blocked IPSCs (data not shown) identifying these currents as GABA_A-mediated IPSCs. When 30 μM DA was added to the bath, IPSC amplitudes were statistically significant reduced to 80 ± 4% of control (P < 0.05; n = 36). Figure 1B shows IPSCs evoked under control conditions and in the presence of DA, scaled to the same peak amplitude. It can be seen that IPSC kinetics were not significantly affected by DA (half rise time: 2.4 ± 0.3 ms, P > 0.9, n = 21 and decay time constant: 24.9 ± 3 ms, P > 0.8, n = 21). The frequency of spontaneous IPSCs was increased during application, as reported previously (Zhou and Hablitz 1999). Washout of DA for 10 min partially reversed the observed effect (Fig. 1A). No significant change in holding current (43.5 ± 3 pA control vs. 41.3 ± 5 pA DA, P > 0.5, n = 36) or IPSC reversal potential (−4.9 ± 3 mV, P > 0.5; n = 5) was observed after DA application. Figure 1C depicts the relationship between stimulation strength and IPSC amplitude. DA inhibited responses at all intensities.

**Concentration dependence of DA effects**

The effect of varying DA concentration on IPSC reduction in PFC pyramidal neurons was tested. As shown in Fig. 2, DA-induced reductions in IPSC amplitude were concentration-dependent between 1 and 100 μM DA. Fitting the experimental points to a Hill function, we obtained an IC_50 value of 30.3 μM. The maximum IPSC reduction was 33.2%. Over this range of concentrations, DA’s effect was monophasic and showed saturation at ~100 μM. No desensitization was observed with continued application of DA or when DA was added repeatedly after washout intervals of ≥10 min (not shown).

**Role of DA receptor subtypes**

To elucidate the receptor subtype mediating DA-induced reductions in IPSCs, the effects of specific DA receptor ago-
nists and antagonists were investigated. Bath application of the D1-like receptor agonist SKF 38393, at a concentration of 10 μM, significantly reduced IPSC amplitude (to 71.5 ± 8% of control P < 0.01, n = 10; Figs. 3A and 4). The reduction with SKF 38393 was not significantly different from that observed with 100 μM DA (67.6 ± 7 vs. 71.5 ± 8%, P > 0.85). In contrast, the D2-like receptor agonist quinpirole (10 μM) had no significant effect (94.5 ± 5%, n = 7, P > 0.5) on IPSC amplitude (Figs. 3C and 4). Neither agonist affected IPSC kinetics because half rise times (2.5 ± 0.1 ms in SKF 38393 and 2.4 ± 0.4 ms in quinpirole; P > 0.8 and P > 0.7, respectively) and decay time constants (27.6 ± 3 ms in SKF 38393 and 22.8 ± 5; P > 0.7 and P > 0.5, respectively) were not significantly changed.

These results indicate D1-like receptors as the subtype activated by DA to produce the observed reductions of IPSC amplitude. To confirm this, we examined the effect of blocking DA receptors with the specific antagonists SCH 23390 (for D1-like DA receptors) and sulpiride (for D2-like DA receptors). The D1- or D2-like antagonists were applied for 20 min before the control responses were evoked. DA was subsequently added in the continued presence of the antagonist. Bath application of SCH 23390 or sulpiride had no effect on IPSC amplitude (103.1 ± 4% for SCH23390, P > 0.5, n = 3 and 98.3 ± 3% for sulpiride, P > 0.5, n = 3) or holding current at −70 mV. Figure 3B shows that blocking D1-like receptors with SCH 23390 (10 μM), prevented the DA-dependent reduction of IPSCs (80.6 ± 5% of the control condition in DA vs. 98.2 ± 4% in DA plus SCH23390; P < 0.05; n = 10). In the presence of sulpiride (20 μM), DA had an inhibitory effect (79.7 ± 4%, P > 0.05, n = 6) on IPSC amplitude similar to that observed in the case of DA alone (Fig. 4). The effects of DA and D1 agonists are summarized in Fig. 4.

FIG. 4. Summary of the effects of DA agonists and antagonists on IPSCs. Addition of DA 30 μM to the bath solution caused a significant (P < 0.01) reduction in IPSC amplitude (81 ± 3% of control amplitude, n = 10). A specific D1 agonist, SKF38393 (10 μM) reduced significantly (P < 0.01) the IPSC amplitude (73.6 ± 8%, n = 10). Blocking D1 receptors with the selective D1 subtype DA receptor antagonist, SCH33930 (10 μM), significantly (P < 0.01) prevented DA reduction of IPSC (98 ± 4%, n = 7). The selective D2 agonist, quinpirole (10 μM), did not significantly reduce IPSC amplitude (94 ± 5%, n = 7, P > 0.5). DA (30 μM), in the presence of the D2 specific antagonist sulpiride (20 μM), still decreased IPSC amplitude (80 ± 4%; n = 7) in cortical pyramidal neurons. Antagonists were present in the bath 15–20 min before obtaining control records and during DA application. No significant change in IPSC amplitude vs. control without antagonist was observed in the presence of either antagonist alone (103 ± 4%; for SCH23390, n = 3 and 98 ± 3.0% for sulpiride, n = 3, P > 0.05). *, significant difference compared with control.

DA appears to decrease IPSCs presynaptically in PFC neurons

To examine whether the inhibitory action of DA on IPSCs is mediated by a presynaptic reduction in GABA release, we studied the effect of DA on the ratio of IPSC amplitudes evoked by paired stimulation. The ratio was calculated by dividing the amplitude of the test response by the control response amplitude. Two stimuli were given at an interpulse interval of 50 ms. The GABA B receptor antagonist SCH 50911 (10 μM) was bath applied to prevent activation of presynaptic autoreceptors. SCH 50911 had no effect on control IPSC amplitude or kinetics (not shown) but abolished paired-pulse depression. In the presence of SCH 50911, no paired-pulse inhibition was observed in our preparation under control conditions as shown in Fig. 5. The paired-pulse ratio was near 1. Addition of 10 μM SKF38393 reduced both control and test IPSCs resulting in a significant decrease in paired-pulse ratios (1.0 ± 0.2 vs. 0.8 ± 0.2; P < 0.05, n = 12), indicating paired-pulse depression. This result suggests that DA is altering presynaptic function.

To rule out postsynaptic changes in GABA responses after DA application, GABA was pressure applied locally. Figure 6A shows representative currents evoked in one PFC pyramidal neuron by GABA before and during application of DA. GABA response amplitude was not significantly changed in 5 cells tested in the presence of DA as can be seen in Fig. 6B (control, 910.5 ± 403 pA vs. DA, 988.6 ± 470 pA, P > 0.5, n = 5).
Effects of the kinase inhibitor H-8

Activation of D1-like receptors is coupled to the activation of the AC-PKA second-messenger pathway. We therefore tested the effect of H-8, a membrane permeable PKA inhibitor. H-8 was bath applied for $20 \text{ min}$ before DA application. Figure 7 shows that H-8 blocked the inhibitory effect of $30 \text{ mM}$ DA on IPSCs in PFC pyramidal neurons. In control condition, $30 \text{ mM}$ DA decreased IPSCs to $79\pm11\%$ of control. DA, applied in the presence of $5 \text{ mM}$ H-8, did not reduce IPSCs ($108\pm10\%$ of control). The DA plus H-8 condition was significantly different from DA ($P<0.05$, $n=5$) but not significantly different from the control without DA ($P>0.6$, $n=5$).

Discussion

The main finding of this study is that DA, acting on D1-like receptors, reduced evoked IPSC amplitudes in layer II/III pyramidal neurons in rat PFC. Concurrently, the frequency of spontaneous IPSCs was increased as described previously (Zhou and Hablitz 1999). The reduction in evoked IPSCs appears to mediate via a presynaptic D1 receptor-mediated effect. DA did not significantly alter passive membrane properties and repetitive firing at the concentration employed. The present results provide evidence that there is a differential DA regulation of spontaneous and evoked IPSCs in rat PFC.

Postsynaptic effects of DA

Although there is a general consensus that DA has a predominantly inhibitory effect on neuronal activity in PFC, the direct effect of DA on the membrane properties of individual PFC neurons is less clear. An early in vivo study showed that a membrane depolarization accompanied DA inhibition of cortical neurons (Bernardi et al. 1982). The number of spikes elicited by depolarizing current pulses in PFC neurons in vitro has been reported to be decreased by low concentrations (0.1–10 $\mu\text{M}$) of DA (Geijo-Barrientos and Pastore 1995). Higher DA concentrations (20–100 $\mu\text{M}$) produce an increase in excitability (Penit-Soria et al. 1987; Shi et al. 1997), perhaps via D1 receptors (Yang and Seamans 1996). DA also has been reported to decrease excitability of layer V PFC neurons via D2 receptor activation (Gulledge and Jaffe 1998). Our previous findings showed DA to have variable effects on layer II/III PFC pyramidal cells while consistently increasing the excitability of GABAergic interneurons (Zhou and Hablitz 1999). The present results are consistent with the idea that layer II/III PFC pyramidal neurons do not display robust postsynaptic responses to DA application.

Mechanism of DA inhibition of IPSCs

Multiple types of DA receptors are expressed in the CNS. At present, five different DA receptor proteins are known to be produced by five distinct genes (Misalle et al. 1998). According to their pharmacological and physiological properties, DA receptors were originally classified broadly into D1 and D2.
types, which are positively and negatively coupled to AC, respectively (Kebabian and Calne 1979). Two major subfamilies are now recognized, the D1- and D2-like receptors. DA modulates a number of Ca$^{2+}$ (Cepeda et al. 1998; Hernández-López et al. 1997; Yan et al. 1997) and K$^+$ currents (Liu et al. 1994; Pedarzani and Storm 1995) in a complex manner dependent on cell type. DA has also been reported to modulate an inward rectifying hyperpolarization-activated current (Jiang and Haddad 1997). More recently, it has been reported that activation of D1-like DA receptors reduce peak Na$^+$ currents in acutely isolated hippocampal neurons through phosphorylation of the alpha subunit of the Na$^+$ channel by PKA activation (Cantrell et al. 1999). In addition, direct modulation of NMDA receptors also reduces peak Na$^+$ currents in striatal neurons are decreased via a D1-PKA-dependent mechanism (Surmeier et al. 1995). Activation of D1-like DA receptors also reduces peak Na$^+$ currents in acutely isolated hippocampal neurons through phosphorylation of the alpha subunit of the Na$^+$ channel by PKA activation (Cantrell et al. 1999). Although Na$^+$ and Ca$^{2+}$ currents are potential DA

DA inhibition of IPSCs was not observed in the presence of H-8, an inhibitor of PKA. This is consistent with the known ability of D1-like receptors to stimulate AC. At higher concentrations, H-8 can inhibit protein kinase C. Because activation of AC-PKA is the principal biochemical response following D1-like receptor activation; it seems likely that H-8 was present in DA 30 m containing bath solution. No significant change (97 ± 15, P > 0.05, n = 5) was observed in IPSC amplitude between control saline solution alone and when H-8 was added and after 15 min of incubation (not shown). *, significant difference compared with control.

FIG. 7. Bath application of 5 μM H-8, a specific inhibitor of PKA, prevents DA reduction of IPSCs in PFC pyramidal neurons (108 ± 11%, P > 0.05; n = 5). H-8 was added 15 min previous to the recording of control currents and was present in DA 30 μM containing bath solution. No significant change (97 ± 15, P > 0.05, n = 5) was observed in IPSC amplitude between control saline solution alone and when H-8 was added and after 15 min of incubation (not shown). *, significant difference compared with control.

Possible effects of AC-PKA

DA receptor activation has been reported to decrease postsynaptic GABA responses in neostriatal neurons (Flores-Hernandez et al. 2000). This was mediated via a PKA/DARPP-32/protein phosphatase effect on GABA receptor β1 subunits. In the present study, changes in response to directly applied GABA were not observed. This could reflect presence of GABA receptors with a different subunit composition, alterations in signaling pathways or differences in the level of DA innervation of pyramidal cells.
targets, the exact mechanism whereby DA reduces IPSPs in neocortical neurons needs to be determined.

In conclusion, we have shown that DA can inhibit IPSPs in layer II/III pyramidal cells. This appears to be via a presynaptic mechanism apparently involving D1-like receptor stimulation of an AC-PKA-mediated process. Spontaneous and evoked IPSPs appear to be differentially regulated by DA. This raises the possibility that DA could decrease overall cortical excitability by enhancing GABAergic tone via an increase in spontaneous IPSCs while facilitating specific inputs by reducing evoked IPSCs.

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REFERENCES


