A Postsynaptic Interaction between Dopamine D_1 and NMDA Receptors Promotes Presynaptic Inhibition in the Rat Nucleus Accumbens via Adenosine Release

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The mechanism underlying dopamine D_1 receptor-mediated attenuation of glutamatergic synaptic input to nucleus accumbens (NAcc) neurons was investigated in slices of rat forebrain, using whole-cell patch-clamp recording. The depression by dopamine of EPSCs evoked by single-shock cortical stimulation was stimulus-dependent. Synaptic activation of NMDA-type glutamate receptors was critical for this effect, because dopamine-induced EPSC depressions were blocked by the competitive NMDA receptor antagonist D/L-2-amino-5-phosphonopentanoate (AP5). Application of NMDA also depressed the EPSC, and both this effect and the dopamine depressions were blocked by the A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), implicating adenosine release in the EPSC depression. A_1 receptor agonists also depressed EPSCs by a presynaptic action, causing increased paired-pulse facilitation, but this was insensitive to AP5. Activation of D_1 receptors enhanced both postsynaptic inward currents evoked by NMDA application and the isolated NMDA receptor-mediated component of synaptic transmission. The biochemical processes underlying the dopamine-induced EPSC depression did not involve either protein kinase A or the production of cAMP and its metabolites, because this effect was resistant to the protein kinase inhibitors H89 and H7 and the cAMP-specific phosphodiesterase inhibitor rolipram. We conclude that activation of postsynaptic D_1 receptors enhances the synaptic activation of NMDA receptors in nucleus accumbens neurons, thereby promoting a transsynaptic feedback inhibition of glutamatergic synaptic transmission via release of adenosine. Unusually for D_1 receptors, this phenomenon occurs independently of adenylyl cyclase stimulation. This process may contribute to the locomotor stimulant action of dopaminergic agents in the NAcc.

Key words nucleus accumbens; whole-cell patch-clamp recording; rat; brain slices; glutamatergic synaptic transmission; presynaptic inhibition; retrograde messenger; dopamine; adenosine; glutamate; dopamine D_1 receptors; NMDA receptors; adenosine A_1 receptors; adenylyl cyclase; cyclic AMP; protein kinase A

The ventral extension of the striatum, the nucleus accumbens (NAcc), is a major projection field of the mesolimbic dopamine system (Deutch and Cameron, 1992). NAcc neurons also receive excitatory glutamatergic inputs primarily from cortical regions and also from the thalamus, hippocampus, and amygdala (see Pennartz et al., 1994). Because the predominant cell type, the GABA-containing medium spiny projection neurons, are generally quiescent in nature, this excitatory drive is extremely important for generation of outputs from the NAcc (Pennartz et al., 1994; Wilson and Kawaguchi, 1996).

Dopamine within the NAcc has been implicated critically in promoting locomotion (Pijnenberg and Van Rossum, 1973) (see also Pennartz et al., 1994; Iversen, 1995), in motivation, behavioral drive, and reward (Fibiger and Phillips, 1986; Robbins and Everitt, 1996), including the behaviors associated with addictive drugs of abuse (Koob, 1992; Kalivas et al., 1993; Wise, 1996), and also in the cognitive dysfunction of schizophrenia (Iversen, 1995; Wan et al., 1995). Interactions between dopaminergic and glutamatergic processes in the NAcc often have been proposed to contribute to these behaviors (Kalivas et al., 1993; Iversen, 1995; Hyman, 1996; Robbins and Everitt, 1996; Wise, 1996). Two functional cellular models for such processes have emerged recently. First, induction of gene expression in striatal neurons by the psychomotor stimulant drug amphetamine depends on activation of both postsynaptic dopamine D_1 and NMDA-type glutamate receptors (Konradi et al., 1996). Second, D_1 receptor activation enhances depolarization of striatal neurons resulting from NMDA application (Levine et al., 1996). D_1 receptor-stimulated adenylyl cyclase (Stoof and Kebabian, 1981) has been implicated in both of these phenomena (Colwell and Levine, 1995; Konradi et al., 1996).

Electrophysiological studies have demonstrated that activation of D_1-like receptors depresses glutamatergic synaptic input to the NAcc (Higashi et al., 1989; Pennartz et al., 1992; Harvey and Lacey, 1996c; Nicola et al., 1996). This is thought to involve a presynaptic mechanism, because dopamine-induced depressions are accompanied by an increase in the degree of paired-pulse facilitation (Pennartz et al., 1992; Nicola et al., 1996) and occur in the absence of any change in the postsynaptic cell membrane potential or conductance (Pennartz et al., 1992; Harvey and Lacey, 1996c; Nicola et al., 1996) (but see Higashi et al., 1989). Furthermore, dopamine also reduces the frequency, but not the amplitude, of miniature EPSCs (Nicola et al., 1996). However,

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this dopamine-induced depression of EPSCs does not seem to involve adenylyl cyclase stimulation (Harvey and Lacey, 1996c).

In contrast to these latter electrophysiological findings, anatomical studies of the striatum provide little support for presynaptic D_1 receptors but demonstrate the existence of both postsynaptic D_1 receptors (Hersch et al., 1995) and the messenger RNA encoding their synthesis (Gerfen et al., 1990; Le Moine and Bloch, 1995). We sought to resolve this discrepancy, and here we demonstrate electrophysiologically that a postsynaptic interaction between D_1 and NMDA receptors results in liberation of a retrograde messenger, which itself inhibits presynaptically the release of glutamate.

Some of these results have been reported previously in abstract form (Harvey and Lacey, 1996a,b).

MATERIALS AND METHODS

Brain slice preparation and recording techniques. Experiments were performed on horizontal slices of ventral forebrain prepared from male Wistar rats 4–5 weeks of age, as described previously (Harvey and Lacey, 1996c). In brief, after inhalational anesthesia (3% Fluothane) animals were decapitated and their brains removed. Horizontal forebrain slices (350 μm thick) were prepared at 4–10°C using a Vibroslice (Campden Instruments, Loughborough, UK). All slices that were used contained or were within 350 μm of the dorsal or ventral extent of the anterior commissure. Slices were maintained in the recording chamber at 32–33°C and superfused continuously at 2–3 ml/min with artificial cerebrospinal fluid comprising (in mM): NaCl 126, KCl 2.5, $NaHCO_3$ 26, $NaHPO_4$ 1.2, $CaCl_2$ 2.4, $MgCl_2$ 1.3, and D-glucose 10, saturated with 95% O_2/5% CO_2 at pH 7.4.

Recordings were obtained using the "blind" whole-cell patch-clamp recording technique (Blanton et al., 1989). Recording pipettes were positioned in the ventral portion of the nucleus accumbens and had resistances of 5–7 MΩ when filled with electrolyte solution comprising (in mM): potassium gluconate 125, $MgCl_2$ 2, NaCl 10, $CaCl_2$ 1, HEPES 10, 1,2-bis(2-aminophenoxy)ethane-*N,N,N′,N′*-tetraacetic acid (BAPTA) 10, adenosine trisphosphate (ATP) 2, and guanosine trisphosphate (GTP) 0.3, buffered to pH 7.2–7.3 with KOH. Membrane potential and current were measured by an Axopatch-1D patch-clamp amplifier (Axon Instruments, Foster City, CA). Series resistance was measured in current clamp, and after electrical compensation (by 70–85%) this ranged from 10 to 22 MΩ. Throughout voltage-clamp recordings input resistance and whole-cell access were monitored via voltage steps (+10 mV, 50 msec) delivered every 20 sec. Neurons that displayed >20% change in the shape or size of capacitance transients or input resistance (in the absence of drugs) were excluded from analysis.

Synaptic currents were evoked by delivering single shocks (0.1 msec, 1–5 mV) every 20 sec, using a bipolar stimulating electrode positioned 300–900 μm rostral to the recording pipette, adjacent to the cerebral cortex. To study solely the glutamate receptor-mediated excitatory postsynaptic currents (Harvey and Lacey, 1996c), we performed all experiments in the presence of picrotoxin (50 μM) to block $GABA_A$ receptor-mediated synaptic currents. Picrotoxin was applied within 5–10 min of obtaining the whole-cell configuration. In all experiments cells were voltage-clamped at between −80 and −90 mV (close to the resting membrane potential), unless otherwise stated (i.e., when NMDA receptor-mediated excitatory synaptic currents were studied).

Data acquisition and analysis, together with generation of voltage and current pulses and timing of electrical stimulation, were performed by pCLAMP software (Axon Instruments). Synaptic currents were stored as the average of five consecutive records, and the peak amplitude of the averaged EPSCs was measured. The percentage of depression/facilitation induced by an agent was expressed relative to the control EPSC amplitude averaged over the 5 min period immediately before drug additions. Numerical data are expressed as mean ± SEM. Statistical analyses were performed by Student's paired *t* test, and all data were significantly different at $p < 0.05$ unless otherwise stated.

Drugs were applied directly to the superfusate in known concentrations, reaching the recording chamber within 15 sec of switching a tap in the perfusion line. Drugs used were 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and D/L-2-amino-5-phosphonopentanoic acid (AP5) (both from Tocris Cookson, Bristol, UK); picrotoxin, adenosine, dopamine, NMDA, tetrodotoxin (TTX), 2-chloropentyl adenosine (CPA), [*R*]-N^6-(2-phenylisopropyl) adenosine (*R*-PIA), and L-nitroarginine (L-NARG) (all from Sigma, Aldrich, UK). Rolipram and 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) were obtained from Research Biochemicals (Natick, MA).

RESULTS

Properties of nucleus accumbens neurons

Whole-cell patch-clamp recordings were obtained from a total of 127 neurons in the ventrolateral nucleus accumbens. After the whole-cell configuration was obtained, the membrane potential was voltage-clamped at −90 mV to enable cell stabilization. Five minutes later the pipette access resistance was measured and optimally compensated in current-clamp mode. Under these conditions the resting membrane potential and input resistance of these neurons were −83 ± 1.6 mV and 108 ± 2.4 MΩ, respectively. These characteristics correspond to those of the medium spiny projection neurons of the NAcc (Pennartz et al., 1992; O'Donnell and Grace, 1993).

The ability of dopamine to depress EPSCs is stimulus-dependent

To test the possibility that the depressant effect of D_1 receptor activation on the EPSC was attributable to an indirect mechanism, we first examined whether the depression of the EPSC by dopamine required glutamatergic synaptic transmission for its expression. Stimulation was ceased during the first 8 min of dopamine application, which was sufficient time to observe a maximal depression by dopamine under normal conditions of stimulation (Harvey and Lacey, 1996c) (see also Fig. 1*B*). When stimulation was resumed with dopamine still present, the EPSC amplitude was initially the same as before the addition of dopamine but declined only on subsequent stimulation, recovering to control levels on washout ($n = 3$; Fig. 1*A*). Thus these data indicate that ongoing glutamatergic synaptic transmission is required for dopamine to depress EPSCs.

Depression of the EPSC by dopamine is dependent on the synaptic activation of NMDA receptors

Previous reports of a facilitatory influence of D_1 receptors on NMDA responses in the dorsal striatum (Cepeda et al., 1993; Colwell and Levine, 1995; Levine et al., 1996) prompted us to examine whether glutamate acting on NMDA receptors was involved in the depressant action of dopamine. In nine cells the competitive NMDA receptor antagonist AP5 (100 μM) reduced the depression induced by dopamine (30 μM) from 47 ± 3.0 to 4.1 ± 1.4% in a reversible manner (Fig. 1*B*). Thus the EPSC depression induced by dopamine seems to be dependent on the synaptic activation of NMDA receptors.

Adenosine is released after activation of NMDA receptors

In support of this indication that synaptic NMDA receptors might be involved in the depression of the EPSC, application of NMDA (20 μM) itself also reversibly depressed EPSC amplitude by 49 ± 1.9% ($n = 8$; Fig. 2*A,B*). One possible consequence of NMDA receptor activation is the formation of nitric oxide (NO; Schuman and Madison, 1994; Garthwaite and Boulton, 1995), which itself can promote release of a variety of neuroactive substances in the striatum (Guevara-Guzman et al., 1994) and modulate synaptic transmission in other parts of the brain by a trans-synaptic action (see Schuman and Madison, 1994; Garthwaite and Boulton, 1995). A role for NO was explored by examining the effect of a specific inhibitor of NO synthase, L-NARG, on the EPSC depressions caused by NMDA application. Application of NMDA

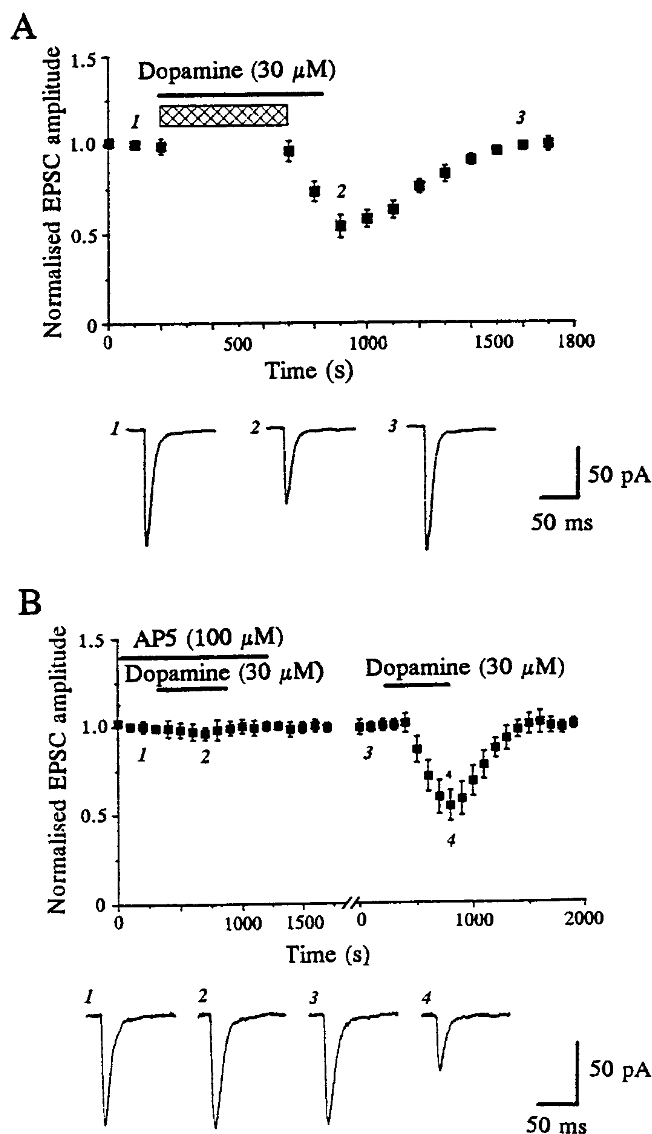

Figure 1. The dopamine-induced depression of the EPSC is dependent on synaptic activation of NMDA receptors. *A*, Single-shock electrical stimulation (to evoke EPSCs) was stopped during the period shown by the *hatched bar*, during which time dopamine (30 μM, *filled bar*) was applied. Only once stimulation was resumed did depression of EPSCs commence. Data were pooled from three separate experiments. Each point on the graph is the average of five consecutive records, such as those shown *below* the graph (taken from a single experiment), and is normalized with respect to the 5 min period immediately before the addition of dopamine. *B*, Depression of EPSCs by dopamine was reversibly blocked by the NMDA receptor antagonist AP5 (100 μM). The plot (*top panel*) illustrates data pooled from four individual neurons, and the *x*-axis break, variable between experiments, represents 10–20 min. *Bottom panel*, Sample records from one experiment, taken at the times indicated.

caused a depression of 49 ± 2.7% ($n = 3$), which was readily reversible on washout (Fig. 2*A*). However, 15–20 min after the addition of L-NARG (100 μM), the ability of NMDA (20 μM) to depress EPSCs was unaffected (mean depression of 48 ± 3.2%; $p > 0.05$; Fig. 2*A*), which suggests that NO is not involved in this process.

Another possible consequence of NMDA receptor activation that might depress the EPSC is the release of adenosine. Adenosine depresses glutamatergic synaptic transmission in many regions of the CNS (for review, see Fredholm, 1995), including the NAcc (Uchimura and North, 1991), and it is released after NMDA receptor activation in cortical tissue (Craig and White, 1993) and also in the hippocampus (Manzoni et al., 1994). The effects of the selective adenosine A_1 receptor antagonist DPCPX (Fredholm, 1995) on NMDA-induced depressions, therefore, were examined. Application of NMDA (20 μM) reduced the amplitude of EPSCs by 34 ± 5.7% ($n = 5$), which was reduced to a depression of only 4.4 ± 2.5% ($n = 5$) in the presence of the DPCPX (200 nM; Fig. 2*B*). This suggests that NMDA receptor activation promotes release of adenosine, which inhibits glutamate release at this synapse via an action on A_1 adenosine receptors.

Depression of the EPSC by dopamine requires activation of adenosine A_1 receptors

Because NMDA receptor activation leads to the release of adenosine and NMDA receptor activation is critical for dopamine-induced depressions of the EPSC, we explored the possible involvement of adenosine in this effect of dopamine, using the selective adenosine A_1 receptor antagonist DPCPX. The ability of dopamine (30 μM) to depress EPSCs was reduced by DPCPX (200 nM) from a depression of 51 ± 3.5 to 2.0 ± 0.8% ($n = 7$; Fig. 2*C*). In a further three cells, depressions induced by the selective D_1 receptor agonist SKF 38393 (10 μM) also were reduced significantly from 47 ± 3.9 to 4.5 ± 1.3% by DPCPX (200 nM). However, in contrast to the actions of dopamine, the ability of the A_1 receptor-selective agonist CPA (200 nM; Fredholm, 1995) to depress EPSCs was unaffected by AP5 (100 μM) in all five cells tested (Fig. 2*D*). Therefore depression of the EPSC by A_1 receptors is itself independent from NMDA receptor activation. Taken together, these findings indicate that the ability of dopamine to depress glutamate receptor-mediated synaptic currents in the nucleus accumbens requires the activation of adenosine A_1 as well as D_1 receptors, and that activation of NMDA receptors is a critical intermediate step for the production of adenosine.

In all cells examined, DPCPX (200 nM) itself caused a rapid and pronounced increase in EPSC amplitude (by 74 ± 14%, $n = 28$; Figs. 2*B,C*, 3*A,B*), which was not accompanied by any discernible change in the holding current or membrane conductance. This effect did not reverse readily on washout of the drug (for up to 30 min). This suggests that glutamate receptor-mediated synaptic transmission is also subject to tonic inhibition by endogenous adenosine, which is relieved by DPCPX.

Adenosine depresses EPSCs by activation of presynaptic adenosine A_1 receptors

The next series of experiments was aimed at establishing the site of action of adenosine at this synapse, because adenosine seems to be critical for dopamine- and NMDA-induced depression of the EPSC. In all 13 cells tested, application of adenosine (30–100 μM) concentration dependently and reversibly depressed the peak amplitude of EPSCs. At a concentration of 60 μM, adenosine depressed the EPSCs by 46 ± 4.4% ($n = 7$; Fig. 3*A*). Depressions were evident 1–2 min after adenosine additions, were sustained for the duration of its application, and reversed within 8–12 min of drug washout. In all 13 cells examined, adenosine caused no significant change in either the holding current or input conductance (measured during the +10 mV voltage step; Fig. 3*A*). Thus, in agreement with Uchimura and North (1991), adenosine (60 μM) depressed glutamatergic synaptic transmission in the nucleus

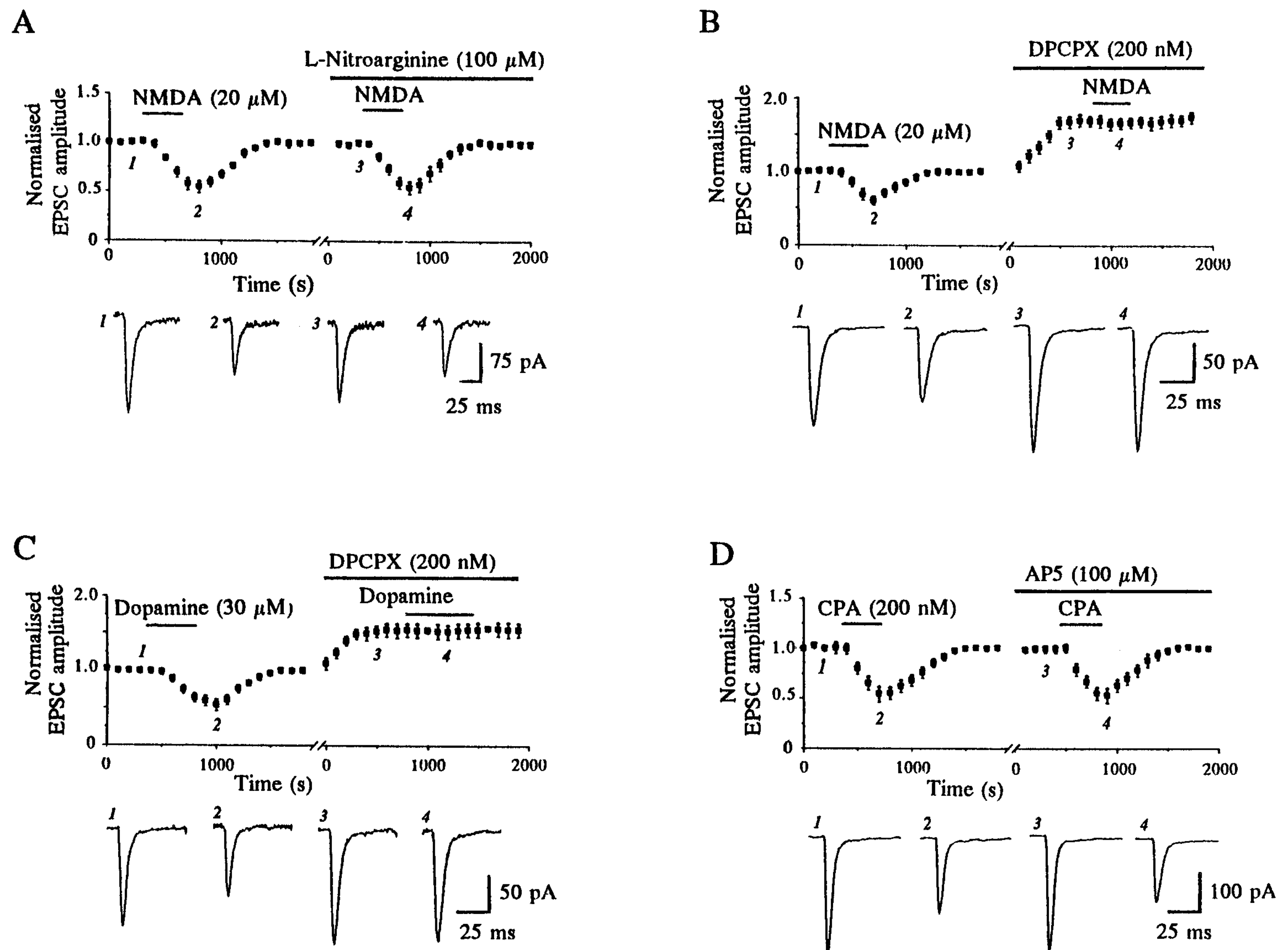

Figure 2. Adenosine A_1 receptor activation is required for EPSC depression by both NMDA and dopamine, indicating that adenosine release results from NMDA receptor activation. *A*, Application of NMDA caused a reversible depression of EPSCs, but this was unaffected by the nitric oxide synthase (NOS) inhibitor L-nitroarginine. The plot shows that NMDA (20 μM) depressed EPSCs in a reversible manner. After perfusion of the NOS inhibitor L-nitroarginine (100 μM) for 15–20 min, reapplication of NMDA caused a similar depression. Data were pooled from three separate experiments, and sample records of EPSCs from one experiment are displayed *below* the plot. *B*, The reversible depression of EPSCs induced by NMDA (20 μM) was reduced considerably by the A_1 receptor antagonist DPCPX (200 nM), which itself increased EPSC amplitude. The plot shows data pooled from five neurons, and the *x*-axis break corresponds to 3–5 min before the addition of DPCPX. *C*, DPCPX also blocks depression of EPSCs by dopamine. Dopamine (30 μM) reduced the amplitude of EPSCs, and this effect was blocked in the presence of DPCPX (200 nM). DPCPX itself caused a clear facilitation of EPSC amplitude. Data were pooled from five individual neurons; the *x*-axis break corresponds to 3–8 min. *D*, The EPSC depression caused by the A_1 receptor agonist CPA (200 nM) is unaffected by the NMDA receptor antagonist AP5 (100 μM). Data were obtained from five experiments; the *x*-axis break corresponds to 10–20 min.

accumbens in the absence of any detectable change in the postsynaptic membrane properties of the cell under study. Similarly, the selective adenosine A_1 receptor agonists (Fredholm, 1995) CPA (200 nM; Figs. 2*D*, 3*B*) and *R*-PIA (200 nM; data not shown) both reversibly depressed the peak amplitude of EPSCs by 55 ± 9.4% ($n = 9$) and 47 ± 6.5% ($n = 8$), respectively, also without any detectable change in postsynaptic membrane conductance. This effect was evident 1–2 min after agonist application and completely reversed 10–12 min after washout. Depressions of EPSCs induced by adenosine (60 μM; $n = 3$), CPA (200 nM; $n = 5$), and *R*-PIA (200 nM; $n = 3$) all were blocked completely by the selective adenosine A_1 receptor antagonist DPCPX (200 nM; Fig. 3*A,B*). Taken together, these data indicate that adenosine depresses EPSCs via an action at A_1 receptors.

To investigate further the locus of action of both applied and endogenous adenosine, we used a paired-pulse stimulation protocol (50 msec interpulse interval). While reducing the amplitude of both EPSCs, adenosine (60 μM) reversibly enhanced the ratio of the second EPSC to the first by 32 ± 3.2% in five cells. In a further five cells CPA (200 nM) also increased the paired-pulse ratio by 98 ± 3.2% (Fig. 3*C*). These findings are consistent with a presynaptic locus for the adenosine A_1 receptors involved in depressing synaptic transmission. Furthermore, the EPSC facilitation induced by DPCPX (200 nM) was accompanied by a decrease of 25 ± 5.1% ($n = 6$) in the corresponding paired-pulse ratio (Fig. 3*D*), indicating that the EPSC depression attributed to endogenous adenosine also is mediated by presynaptic A_1 receptors.

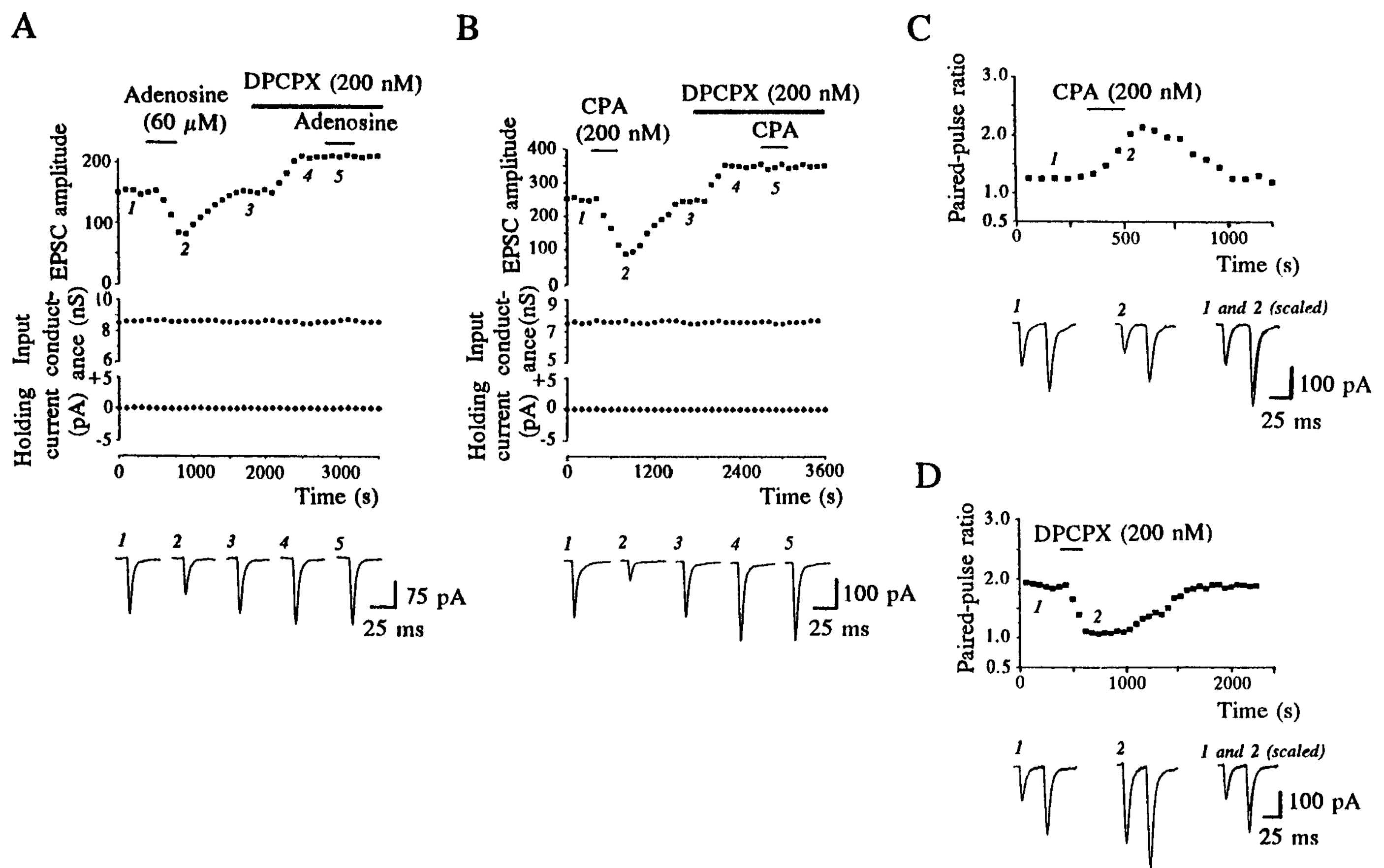

Figure 3. Both endogenous and exogenous adenosine depressed EPSCs via activation of presynaptic adenosine A_1 receptors. *A*, The ability of adenosine to depress EPSCs is blocked by DPCPX (200 nM). The *upper graphs* are plots of EPSC amplitude (*squares*, *top graph*), input conductance (*circles*, *middle graph*), and holding current (*diamonds*, *bottom graph*) during an experiment on a single neuron, voltage-clamped at −90 mV. The *lower panel* shows synaptic currents evoked at specific points (*1–5*) during the same experiment. Neither the reduction in synaptic transmission induced by adenosine nor the enhancement by DPCPX was accompanied by any change in the holding current or input conductance of the neuron. *B*, Similarly, the selective A_1 receptor agonist CPA depressed EPSCs in a DPCPX-sensitive manner. CPA (200 nM) depressed EPSCs (*squares*, *top graph*) in the absence of any effect on the input conductance (*circle*, *middle graph*) or the holding current (*diamond*, *bottom graph*). Data were obtained from a single neuron voltage-clamped at −90 mV. *C*, Paired-pulse facilitation is increased when EPSCs are depressed by the A_1 receptor agonist CPA. CPA (200 nM) produced a reversible enhancement in the paired-pulse ratio evoked with a 50 msec interstimulus interval. The pairs of EPSCs in the *lower panel* were obtained in control conditions (*1*) and in the presence of 200 nM CPA (*2*). In the *right trace* the first EPSC in *2* has been scaled to match the size of the first EPSC in *1*. *D*, Paired-pulse facilitation is decreased when EPSCs are facilitated by the A_1 receptor antagonist DPCPX (200 nM). Thus the depressant actions of both endogenous and applied adenosine are attributable to a presynaptic mechanism.

Postsynaptic potentiation of NMDA currents by D_1 receptor activation

Having established that synaptically activated NMDA receptors are a critical link between the activation of D_1 receptors and the release of adenosine that results in presynaptic inhibition, we then sought to determine more directly whether dopamine could modulate postsynaptic NMDA receptors in the NAcc. This was explored initially by applying dopamine in conjunction with a submaximal dose of NMDA. In three cells voltage-clamped between −80 and −90 mV, inward currents evoked in response to application of NMDA (20 μM) were enhanced by 60 ± 4.7% by coapplication with dopamine (30 μM). In the presence of TTX (200 nM), which completely blocked synaptic transmission (data not shown), the selective D_1 receptor agonist SKF 38393 (10 μM) also caused an enhancement (by 71 ± 7.9%, n = 5; Fig. 4) of NMDA receptor-mediated inward currents that was readily reversible on washout in the three cells examined. Thus these data indicate that dopamine acting on D_1 receptors facilitates postsynaptic NMDA receptor-mediated currents, as shown previously in the dorsal striatum (Cepeda et al., 1993; Levine et al., 1996).

Dopamine enhances the NMDA receptor-mediated component of the EPSC

To see whether dopamine could modulate NMDA-induced currents evoked by synaptic activation, as well as those caused by NMDA application, we examined the effect of dopamine on the pharmacologically isolated NMDA receptor-mediated component of synaptic transmission ($EPSC_N$). When cells were voltage-clamped at −50 mV in the presence of CNQX (10 μM), the antagonist of AMPA-type glutamate receptors, single-shock electrical stimulation resulted in a slower synaptic current that was blocked completely by AP5 (100 μM) in all four cells tested (Fig. 5*A*). In a manner similar to its action on fast AMPA receptor-mediated synaptic currents, dopamine (30 μM) reversibly depressed the peak amplitude of the $EPSC_N$ by 51 ± 3.1% (n = 8; Fig. 5*B*). However, in the presence of DPCPX (200 nM), dopamine (30 μM) caused a clear and readily reversible enhancement of $EPSC_N$ amplitude (by 28 ± 4.5%) in all nine cells examined (Fig. 5*B*). The adenosine A_1 receptor antagonist DPCPX itself caused a rapid facilitation of the $EPSC_N$ amplitude (by 74 ± 12%, n = 9; Fig. 5*B*), similar to that observed with the EPSC (e.g., Fig.

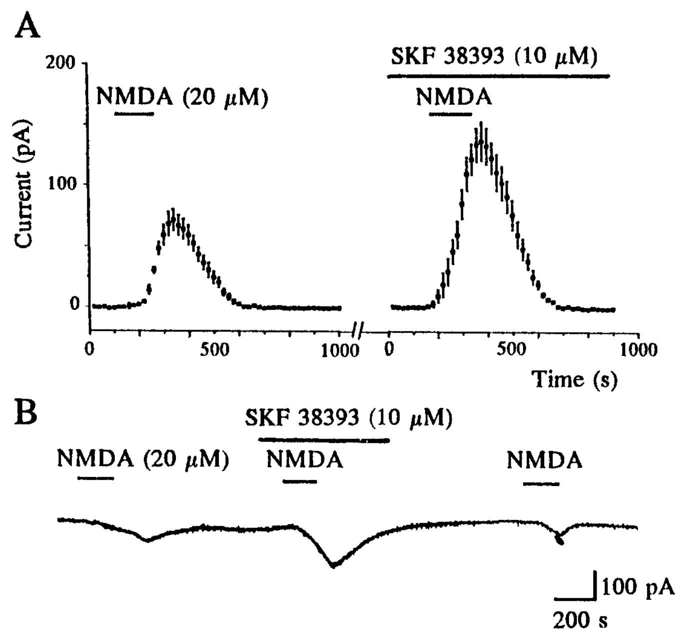

Figure 4. Dopamine D_1 receptor activation enhances postsynaptic NMDA receptor-mediated inward currents independently of synaptic transmission. *A,* Data pooled from five cells showing that bath application of NMDA (20 μM) induced an inward current. When NMDA was reapplied in the same five cells 5–10 min after application of the D_1 receptor agonist SKF 38393 (10 μM), NMDA-induced currents were enhanced. Cells were voltage-clamped at −80 to −90 mV with tetrodotoxin (200 nM) present throughout. *B,* Continuous record of membrane current from an individual neuron (1 of the 5 in *A*; voltage-clamped at −90 mV) showing the reversible enhancement of the NMDA-mediated current by SKF 38393 (10 μM).

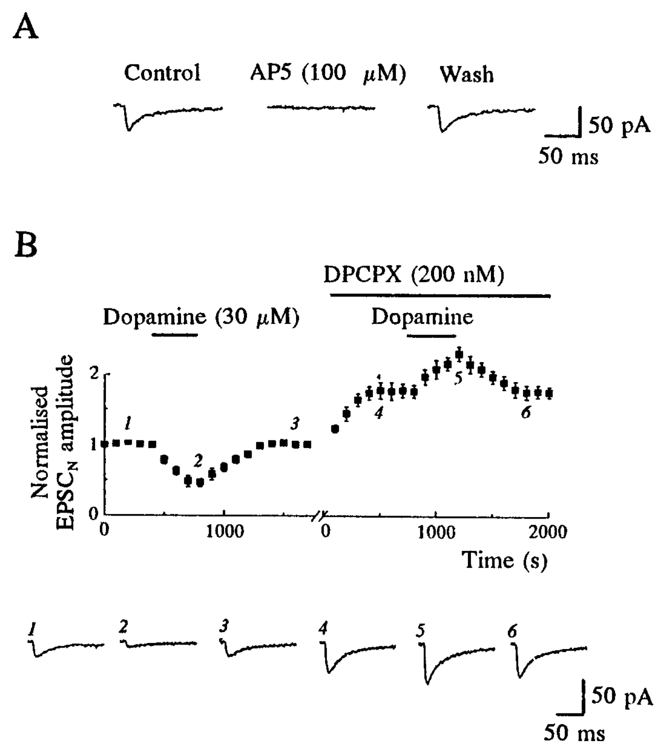

Figure 5. Adenosine A_1 receptor blockade prevents dopamine-mediated depression of NMDA receptor-mediated EPSCs ($EPSC_N$) and reveals that dopamine enhances the $EPSC_N$. *A,* The $EPSC_N$ was isolated by application of CNQX (10 μM) to block AMPA receptors and by voltage-clamping at −50 mV. Under these conditions the residual component of the EPSC was blocked completely by the NMDA receptor antagonist AP5 (100 μM). *B, Top panel,* Dopamine (30 μM) reversibly reduced the $EPSC_N$ amplitude. In the presence of the A_1 receptor antagonist DPCPX (200 nM), which itself increased $EPSC_N$ amplitude, dopamine caused a clear and reversible facilitation of the $EPSC_N$. The plot shows data pooled from nine cells, and the *x*-axis break corresponds to 3–8 min before the addition of DPCPX. *Bottom panel,* Sample records from one experiment, taken at the times indicated on the plot.

2*B*). These data suggest that, in the absence of the inhibitory action of endogenous adenosine, dopamine facilitates, rather than depresses, the isolated NMDA receptor-mediated component of synaptic transmission. This is likely to be attributable to a postsynaptic interaction between D_1 and NMDA receptors.

Biochemical mechanism of the depression of the EPSC by dopamine

It is well established that striatal D_1 receptors couple to adenylyl cyclase, and their activation results in the stimulation of cAMP formation and subsequent activation of protein kinase A (PKA; Stoof and Kebabian, 1981). However, stimulation of adenylyl cyclase with forskolin and elevation of intracellular cAMP levels with either dibutyryl cAMP or inhibitors of cAMP-specific phosphodiesterases such as IBMX or rolipram caused facilitation, rather than depression, of the EPSC (Harvey and Lacey, 1996c). Therefore we have proposed that D_1 receptor-mediated inhibition of glutamatergic synaptic transmission in the nucleus accumbens does not involve a cAMP-dependent process (Harvey and Lacey, 1996c). Furthermore, in the presence of the PKA inhibitor H-89 (1 μM), dopamine (30 μM) depressed the EPSC by 49 ± 3.3%, which was not significantly different from the depression (48 ± 3.9%) evoked in control conditions ($n = 4$; $p > 0.05$; Fig. 6*A*). H-89 (1 μM) was clearly active in this system, however, because it prevented the increase in EPSC amplitude by forskolin (10 μM; $n = 3$; Fig. 6*A*). This particular effect of forskolin has been described previously (Colwell and Levine, 1995; Harvey and Lacey, 1996c) and attributed to PKA-dependent phosphorylation of postsynaptic neostriatal AMPA receptors (Colwell and Levine, 1995). Similarly, dopamine (30 μM) depressed synaptic currents by 42 ± 3.0 and 43 ± 2.6% ($n = 3$, $p > 0.05$) in the presence and absence of the nonselective protein kinase inhibitor H-7 (10 μM), respectively. Thus these data provide further evidence that a PKA-independent process underlies the EPSC depression induced by dopamine.

In other brain regions presynaptic inhibition of synaptic transmission after metabotropic receptor activation has been attributed to adenosine produced by the metabolism of cAMP by cAMP-specific phosphodiesterases (Gereau and Conn, 1994; Bonci and Williams, 1996). If the formation of cAMP and its subsequent metabolism was the principal biochemical process underlying dopamine-induced depressions of the EPSC, then rolipram, a specific inhibitor of cAMP-specific phosphodiesterase (Beavo and Reifsnyder, 1990), might be expected to alter the ability of dopamine to reduce synaptic currents. However, although rolipram (10 μM) caused a rapid enhancement in EPSC amplitude (by 26 ± 4.3%), as reported previously (Harvey and Lacey, 1996c), it did not alter the ability of dopamine to depress synaptic transmission ($n = 3$; Fig. 6*B*). Moreover, when the facilitatory action of forskolin on the EPSC was abolished completely by H-89 (1 μM; $n = 3$), no other effects of forskolin were observed (Fig. 6*A*), although production of cAMP would be

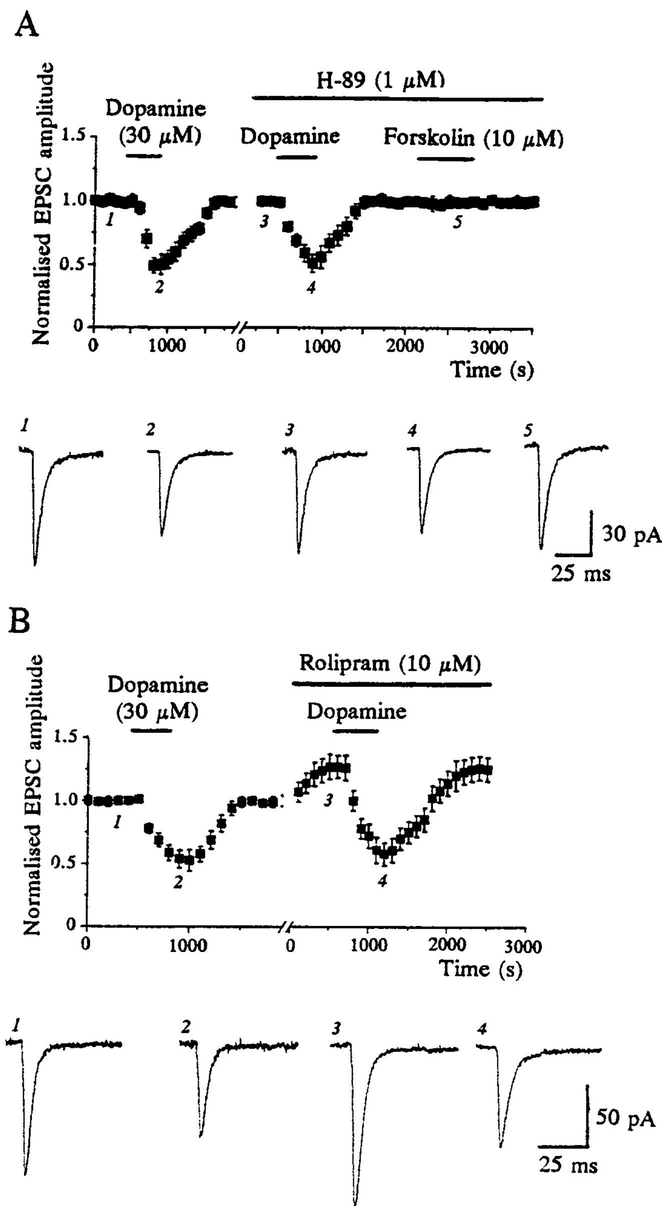

Figure 6. Dopamine depresses EPSCs independently of protein kinase A or metabolism of cAMP. *A*, Dopamine-induced depressions were unaffected by the protein kinase A inhibitor *H-89* (1 μM). However, the ability of forskolin (10 μM) to enhance EPSCs was blocked by *H-89*. The plot consists of data pooled from three cells, and the *x*-axis break corresponds to 3–5 min. *B, Rolipram* (10 μM), a cAMP-dependent phosphodiesterase inhibitor, failed to affect dopamine-induced depressions. The data in the plot are pooled from three cells, and the *x*-axis break corresponds to 3–6 min.

unimpaired under these conditions. Together these findings do not support a role for cAMP, PKA, or a metabolite of cAMP in the dopamine-induced depression of the EPSC.

DISCUSSION

We have shown that the depression of glutamatergic synaptic transmission in the NAcc by dopamine involves a novel indirect process, central to which is an interaction between D_1 and NMDA receptors and subsequent adenosine release. Adenosine in turn acts as a retrograde messenger and inhibits glutamate release via activation of presynaptic adenosine A_1 receptors (Fig. 7). This mechanism argues against the presynaptic localization of D_1 receptors proposed in previous electrophysiological studies (Pennartz et al., 1992; Harvey and Lacey, 1996c; Nicola et al., 1996), but not the anatomical evidence for postsynaptic D_1 receptors in the striatum (Hersch et al., 1995).

A pivotal role for adenosine A_1 receptors in the presynaptic inhibition of glutamatergic synaptic transmission

EPSC depressions induced by dopamine and the selective D_1 receptor agonist SKF 38393 and also by NMDA were abolished by the A_1 receptor antagonist DPCPX. In agreement with a previous report (Uchimura and North, 1991), adenosine reversibly depressed excitatory synaptic transmission in the NAcc in all cells examined. This was attributable to activation of A_1 receptors, because the selective adenosine A_1 receptor agonists CPA and *R*-PIA both mimicked the actions of adenosine, and DPCPX, a specific adenosine A_1 receptor antagonist, completely blocked depressions induced by all the A_1 receptor agonists.

In agreement with Uchimura and North (1991), A_1 receptor agonists at concentrations capable of depressing EPSCs had no discernible effect on the postsynaptic membrane properties (holding current or input conductance) of NAcc neurons. In addition, depressions induced by adenosine and CPA were accompanied by an increase in the paired-pulse ratio, whereas a reduction in the degree of paired-pulse facilitation accompanied the EPSC augmentation induced by DPCPX. These changes in paired-pulse ratio indicate a presynaptic locus for the A_1 receptors regulating glutamatergic synaptic transmission in the NAcc. Although A_1 receptor mRNA has been located in a minority of striatal neurons (Ferré et al., 1996), postsynaptic A_1 receptors play no obvious role in the effects of adenosine, NMDA, and dopamine described here. Thus glutamate release is likely to be inhibited presynaptically not by D_1 receptors, but by A_1 receptors, which are activated indirectly by dopamine.

NMDA receptor activation is critical for the effect of dopamine

Synaptic activation of NMDA receptors is critical for dopamine-induced EPSC depressions, because the ability of dopamine to depress glutamatergic EPSCs is both stimulus-dependent and blocked by the competitive NMDA receptor antagonist AP5. Thus it follows that there must be significant activation of NMDA receptors in the presence of dopamine, although NMDA receptors apparently contribute little to the EPSC (Harvey and Lacey, 1996c) (see also Figs. 1*B*, 2*D*). However, glutamatergic synaptic potentials (EPSPs) in NAcc neurons do exhibit an NMDA component (Martin et al., 1997), particularly at membrane potentials less negative to −80 mV. Therefore a significant activation of synaptic NMDA receptors in neurons other than the (voltage-clamped) cell under study probably occurs under our experimental conditions. The postsynaptic facilitatory interaction between D_1 and NMDA receptors was demonstrated by the potentiation by dopamine and the selective D_1 receptor agonist SKF 38393 of inward currents evoked by applied NMDA and also was demonstrable on the $EPSC_N$. Similar findings have been reported in dorsal striatal neurons (Cepeda et al., 1993; Colwell and Levine, 1995; Levine et al., 1996), and they also resemble in some respects the augmentation of the $EPSP_N$ in NAcc by μ-opioid receptor agonists (Martin et al., 1997). This postsynaptic D_1/NMDA receptor interaction in medium spiny projection neurons constitutes

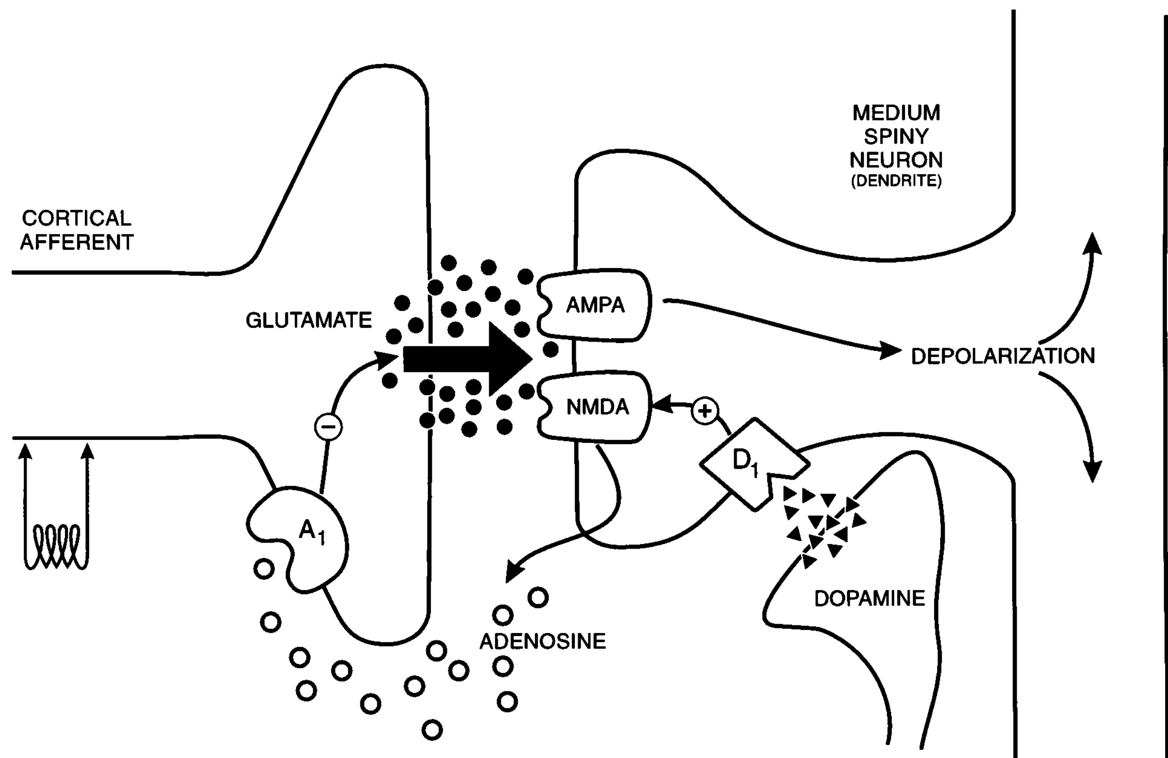

Figure 7. Diagram of a glutamatergic synapse onto a dendritic spine on a medium spiny NAcc output neuron, illustrating the processes operating to permit presynaptic modulation of glutamate release by postsynaptic dopamine D_1 receptors. Glutamate released from the cortical afferent (*left*) activates postsynaptic glutamatergic AMPA and NMDA receptors, resulting in the EPSC. Concurrent activation of D_1 receptors amplifies the current caused by the synaptic activation of NMDA receptors, thereby promoting release of adenosine (or a precursor) into the extracellular space. Adenosine in turn acts on presynaptic inhibitory A_1 receptors to reduce glutamate release. This sequence of events occurs independently of adenylyl cyclase stimulation and production of cyclic AMP.

an attractive candidate mechanism for promoting the EPSC depression.

NMDA itself, as well as D_1 receptor agonists, depressed EPSCs in a DPCPX-sensitive manner, also supporting a role for NMDA receptors "downstream" of the D_1 receptor. In the absence of dopamine, NMDA receptor-dependent release of adenosine during single-shock stimulation seems not to occur, because AP5 does not alter the EPSC amplitude (Harvey and Lacey, 1996c). However, higher frequencies of stimulation, which probably lead to greater NMDA receptor activation, do induce a short-term A_1 receptor-dependent depression of glutamatergic synaptic transmission in the dorsal striatum (Lovinger and Choi, 1995). A similar phenomenon, attributable to NMDA receptor-dependent adenosine release, occurs in the hippocampus (Manzoni et al., 1994). In the NAcc, dopamine seems to enhance sufficiently the level of NMDA receptor activation during single-shock stimulation to promote the release of adenosine.

Release of adenosine after NMDA receptor activation

The precise mechanism whereby extracellular adenosine levels become raised by NMDA receptor activation is unclear. In slices of cortical tissue NMDA causes Ca^{2+}-dependent release of a substrate for ecto-5′-nucleotidase, which then is converted to adenosine (Craig and White, 1993). The inhibitory tone revealed by the EPSC enhancement by DPCPX is unlikely to be NMDA receptor-dependent, however, because AP5 was without effect on EPSC amplitude. Because adenosine may be extruded actively from neurons (Brundege and Dunwiddie, 1996) and formed from a variety of precursors, including ATP, which may itself be released by electrical stimulation (Hamann and Attwell, 1996), there are several possible sources for this endogenous adenosine tone.

Biochemical mechanism coupling D_1 receptors to depression of the EPSC

We have found no evidence to suggest that the D_1 receptor-mediated attenuation of EPSCs involves a cAMP- or PKA-dependent process or requires metabolism of cAMP to adenosine (Harvey and Lacey, 1996c; present study). This perhaps is unexpected, given the considerable evidence for D_1 receptor-stimulated adenylyl cyclase in striatum (Stoof and Kebabian, 1981). Indeed, the observation that forskolin potentiates both NMDA and AMPA receptor-mediated depolarizations and excitatory synaptic potentials in the neostriatum (Colwell and Levine, 1995) certainly suggests a possible role for D_1-stimulated adenylyl cyclase in the NAcc. Nonetheless, although we also observed a PKA-dependent augmentation of the EPSC with forskolin, this was not observed with dopamine, even when feedback inhibition was blocked by DPCPX. Therefore, although regulation of postsynaptic striatal glutamate receptors via PKA may indeed be possible, any stimulation of adenylyl cyclase resulting from D_1 receptor activation was without effect in our experimental paradigm. The effects of dopamine we did observe, which most likely stem from the enhancement of NMDA receptor currents, must arise from a PKA-independent mechanism.

Several recent reports point to the possibility of NMDA current enhancement by neurotransmitter receptors coupled to phospholipase C (PLC; Ben-Ari et al., 1992; Markram and Segal,

1992; Harvey and Collingridge, 1993; Rahman and Neuman, 1996; Pisani et al., 1997). PLC activation by D_1-like receptors has been reported in the striatum (Mahan et al., 1990; Wang et al., 1995), renal tissue (Felder et al., 1993; Yu et al., 1995), and retinal cells (Rodrigues and Dowling, 1990), and this may underlie the dopamine-induced enhancement of NMDA currents in dorsal and ventral striatum (Cepeda et al., 1993; Levine et al., 1996; present study). Alternatively, production of arachidonic acid, also associated with D_1 receptor activation (Piomelli et al., 1991), may increase NMDA-induced currents (Miller et al., 1992).

Physiological and behavioral significance

D_1 receptor activation can induce expression of immediate-early genes in the striatum and nucleus accumbens via NMDA receptor-dependent means (Keefe and Gerfen, 1996; Konradi et al., 1996; Wang and McGinty, 1996), and this may contribute to the long-term plastic changes underlying the behavioral sensitization and dependence associated with addictive drugs (Hyman, 1996). However, because these processes involve D_1 receptor-stimulated adenylyl cyclase and cAMP-dependent phosphorylation, the effects observed in this study may not be related to these behavioral changes.

Postsynaptic NMDA receptors contribute significantly to the regulation of synaptic strength within the NAcc and also play a role in the direct excitation of projection neurons. Although coincident activation of D_1 and NMDA receptors serves initially to enable this process, it is limited subsequently by the reduction in glutamate release resulting from the retrograde action of adenosine. However, because inhibitory GABAergic synaptic transmission in NAcc, which is driven at least partly by glutamatergic synaptic input onto intrinsic GABAergic NAcc neurons, also is depressed by D_1 receptor activation (Pennartz et al., 1992), the net effect of dopamine on the synaptic drive onto NAcc neurons is harder to evaluate. Perhaps because of this, the present findings are not integrated easily with behavioral studies of dopamine/glutamate interactions in NAcc.

Intra-accumbal NMDA receptor antagonists block the orofacial stereotypy (Kelley and Delfs, 1994) and locomotor activity (Pulvirenti et al., 1991; Burns et al., 1994) produced by dopaminergic drugs, mirroring the D_1/NMDA receptor interaction observed in this study. In contrast, NMDA receptor antagonists in the NAcc mimic dopaminergic function in reward (Carlezon and Wise, 1996). Moreover, although dopamine receptor antagonists in the NAcc may be beneficial in schizophrenia (Wan et al., 1995), NMDA receptor antagonists tend to promote psychosis (see Iversen, 1995). Indeed, glutamate receptor agonists, rather than antagonists, have been suggested as an alternative therapy to dopamine receptor antagonists in the treatment of schizophrenia (Carlsson and Carlsson, 1990). Clearly, our understanding of how regulation of NAcc neurons might influence behavior is incomplete (see Pennartz et al., 1994). However, the present cellular and molecular model of an interaction between dopaminergic and glutamatergic neurotransmission in the NAcc, which contains both synergistic and antagonistic components, begins to account for such interactions observed in behavioral studies.

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