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D_1 dopamine receptor stimulation increases GluR1 phosphorylation in postnatal nucleus accumbens cultures

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Abstract

Postsynaptic interactions between dopamine and glutamate receptors in the nucleus accumbens are critical for acute responses to drugs of abuse and for neuroadaptations resulting from their chronic administration. We tested the hypothesis that D_1 dopamine receptor stimulation increases phosphorylation of the AMPA receptor subunit GluR1 at the protein kinase A phosphorylation site (Ser845). Nucleus accumbens cell cultures were prepared from postnatal day 1 rats. After 14 days in culture, GluR1 phosphorylation was measured by western blotting using phosphorylation site-specific antibodies. The D_1 receptor agonist SKF 81297 increased Ser845 phosphorylation in a concentration-dependent manner, with marked increases occurring within 5 min. This was prevented by the D_1 receptor antagonist SCH 23390 and the protein kinase A inhibitor H89, and reproduced by forskolin. The D_2 receptor agonist quinpirole attenuated the response to D_1 receptor stimulation. Neither D_1 nor D_2 receptor agonists altered GluR1 phosphorylation at Ser831, the site phosphorylated by protein kinase C and calcium/calmodulin-dependent protein kinase II. In other systems, phosphorylation of GluR1 at Ser845 is associated with enhancement of AMPA receptor currents. Thus, the present results suggest that AMPA receptor transmission in the nucleus accumbens may be augmented by concurrent D_1 receptor stimulation.

Keywords: AMPA receptors, cell culture, drug addiction, glutamate, phosphorylation, protein kinase A.

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The nucleus accumbens occupies a key position in the neural circuitry of motivation and reward. The two major cell types in the nucleus accumbens are interneurons (10%) and medium spiny GABAergic neurons (90%) (Meredith and Totterdell 1999). The latter are the output neurons of the nucleus accumbens and receive convergent synapses from dopamine (DA) and glutamate terminals (Sesack and Pickel 1990). The DA inputs originate from the ventral mesencephalon, while the excitatory glutamatergic inputs originate from prefrontal cortex and from limbic regions, such as the ventral subiculum region of the hippocampus and the basolateral nucleus of the amygdala. Medium spiny neurons integrate these signals and send projections to motor regions in the ventral pallidum and ventral mesencephalon (reviewed by Groenewegen *et al*. 1999; Kelley 1999). Based on this connectivity and on functional studies, Mogenson (1987) suggested that 'an accumbens dopamine-dependent gating mechanism appears to have a role in translating the motivational determinants of behavior, mediated by the limbic system, into actions…'

Recent work has focused on the role of the nucleus accumbens in drug addiction. Postsynaptic interactions between DA and glutamate receptors on medium spiny neurons are critical for both acute and chronic effects of cocaine and amphetamine (White and Kalivas 1998; Wolf 1998). Electrophysiological studies suggest that DA receptors exert neuromodulatory effects on glutamate-mediated synaptic responses (see Discussion). However, relatively little is known about the cellular mechanisms that enable activation of one receptor family to influence the function of the other. Recent studies in the dorsal striatum suggest that one mechanism may involve regulation of glutamate receptor phosphorylation by DA receptors (Blank *et al*. 1997; Snyder *et al*. 1998; Price *et al*. 1999; Yan *et al*. 1999; Snyder *et al*. 2000).

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Abbreviations used: DA, dopamine; LTD, long-term depression; LTP, long-term potentiation; PKA, protein kinase A.

The present study focused on the question of whether D_1 receptor stimulation regulates phosphorylation of the AMPA receptor subunit GluR1 in primary cultures prepared from postnatal rat nucleus accumbens. GluR1 is expressed by most medium spiny neurons in the adult striatal complex (Bernard *et al*. 1997; Chen *et al*. 1998) and in our primary culture system (Chao *et al*. 1999). Protein kinase A (PKA) phosphorylates GluR1 at Ser845, while calcium/calmodulin-dependent protein kinase II and protein kinase C phosphorylate Ser831 (Roche *et al*. 1996; Barria *et al*. 1997; Mammen *et al*. 1997). Phosphorylation by PKA enhances AMPA receptor currents (Greengard *et al*. 1991; Wang *et al*. 1991; Blackstone *et al*. 1994; Rosenmund *et al*. 1994; Roche *et al*. 1996; Banke *et al*. 2000). Recently, D_1 receptors, which stimulate PKA activity, have been reported to enhance AMPA receptor currents in dorsal striatal neurons via a PKA-dependent mechanism (Price *et al*. 1999; Yan *et al*. 1999; Snyder *et al*. 2000). Since D_1 receptors in the nucleus accumbens stimulate PKA activity, we hypothesized that D_1 receptor activation would lead to increased phosphorylation of GluR1 at Ser845. This was tested using phosphorylation site-specific antibodies to assess GluR1 phosphorylation (Mammen *et al*. 1997).

Materials and methods

Postnatal nucleus accumbens cultures

Postnatal nucleus accumbens cells were cultured according to methods modified from Shi and Rayport (1994). Postnatal (P1) rats were anesthetized by hypothermia. Brains were removed into ice-cold phosphate-buffered saline (PBS). The forebrain was split sagitally at the midline. With the medial surface facing up, a 16G sharp-edged cannula was used to punch out a cylinder of tissue containing the accumbens, using the anterior commissure to define its peripheral border (caudal/dorsal). The lateral one-third of the cylinder (cortex) and the medial one-quarter (using the lateral ventricle as a landmark) of the cylinder were removed using a scalpel blade. The middle portion was transferred to ice-cold CMF solution (calcium- and magnesium-free PBS with 0.5% gentamicin, 1% fungizone and 0.5% glucose) and bubbled with O_2 continuously. Tissue was rinsed with cold CMF and dissociated using papain (20–25 units/mL; Worthington, Lakewood, NJ, USA) for 15–20 min at 37 C, followed by trituration with a Pasteur pipette and a 22G needle. Cells were filtered through fetal bovine serum before plating at 400 000 cells/mL (120 000 cells/cm^2) on poly-D-lysine-coated culture plates with B27 (1/50; Gibco, Grand Island, NY, USA) supplemented NeuroBasal growth medium (Gibco; 2 mM glutamine, 0.5% gentamicin, and 25 μM glutamate). Medium was replaced with glutamate-free NeuroBasal growth medium 24 h later. Then, half of the medium was replaced every 4 days. Experiments were performed after approximately 2 weeks in culture.

Western blots

Cultures were scraped into ice-cold buffer containing 20 mM $NaPO_4$ (pH 7.2), 150 mM NaCl, 10 mM EDTA, 10 mM EGTA, 10 mM NaPPi, 50 mM NaF, 1 mM Na_3VO_4, 1 μM okadaic acid in dimethyl sulfoxide (DMSO), and 1/200 Protease Inhibitor Cocktail Set III (Calbiochem, San Diego, CA, USA). Homogenates were obtained by sonicating culture suspensions three times for 5 s each time on ice and centrifuging at 12 000 *g* for 5 min. Crude membrane pellets were resuspended in homogenization buffer and centrifuged at the same speed again. Protein concentration was determined by the Bio-Rad assay. Samples were loaded onto 8% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) gels (5–10 μg protein/lane). Preliminary experiments demonstrated that these amounts were in the linear range as determined by densitometric analysis (data not shown). Gels were transferred to Hybond ECL nitrocellulose membrane (Amersham, Piscataway, NJ, USA) for immunoblotting.

Immunoblot analysis

Nitrocellulose membranes were rinsed in distilled H_2O and in 0.1% Tween-20 in PBS (PBS-T). They were then blocked with 2% bovine serum albumin for 1–3 h at room temperature, incubated with the phosphorylation site-specific antibodies (P-845, 1/300; P-831, 1/1000) or other antibodies overnight at 4 C, washed with PBS-T, incubated for 60 min with horseradish peroxide-conjugated anti-rabbit IgG (1/10 000; Chemicon, Temecula, CA, USA), and washed. After final washes in PBS-T, membranes were rinsed in PBS, immersed in chemiluminescence (ECL) detecting reagent (for 60 s) and exposed to HyperFilm ECL film (for 5 s to 20 min). Phosphorylation site-specific antibodies were stripped from the membrane by incubating in 62.5 mM Tris (pH 6.8), 2% SDS, and 100 mM 2-mercaptoethanol at 65 C for 60 min. Blots were then probed with a phosphorylation-independent antibody to GluR1 (1/200; Chemicon) to verify that changes in P-845 GluR1 are not attributable to a change in total GluR1 levels. Blots were also probed with antibody to actin (1/5000; Calbiochem, San Diego, CA, USA) as a loading control. The optical density of each band was determined using MOLECULAR ANALYST software (BioRad, Hercules, CA, USA). The relative amount of GluR1 phosphorylation is expressed as the ratio of the signal obtained with the phosphorylation site-specific GluR1 antibody to that obtained with the phosphorylation-independent GluR1 antibody (P-845/GluR1) or with antibody to actin (P-845/actin). Groups were compared by Kruskal–Wallis one-way ANOVA on Ranks followed by a Tukey test (for multiple comparisons) or a Dunnett's test (for comparison of experimental groups to control group). Significance was set at $p < 0.05$.

Results

Time–course and concentration-dependence of D_1 receptor-mediated GluR1 phosphorylation

To determine the effect of D_1 receptor stimulation on phosphorylation of the AMPA receptor subunit GluR1, we added the D_1 receptor selective agonist SKF 81297 to nucleus accumbens cultures, extracted membrane protein, and performed western analysis using an antibody recognizing GluR1 phosphorylated at the PKA site (P-845 antibody; Mammen *et al*. 1997). The total amount of GluR1 was

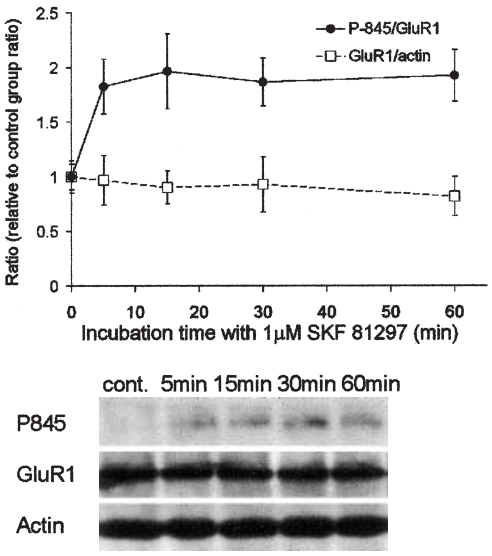

Fig. 1 The D_1 receptor agonist SKF 81297 produced a rapid increase in GluR1 phosphorylation at the PKA site. SKF 81297 was added to cultures for the indicated time at a concentration of 1 μM. Cultures were homogenized and membrane protein was extracted for western analysis with antibody selective for GluR1 phosphorylated at the PKA site (P-845). Blots were also probed with a phosphorylation-independent antibody to measure total GluR1 and with antibody to actin. The ratios of P-845/total GluR1, P-845/actin and total GluR1/actin were determined for each sample and normalized to the control group ratio. Triplicates were run for each condition and data are plotted as the mean ± SEM. Analysis of P-845/GluR1 data using a Kruskal–Wallis one-way ANOVA on ranks indicated no significant effect of time ($F_{4,10} = 2.66$, $p = 0.10$) because the effect was maximal at the first incubation time. However, a *t*-test indicated a significant difference between the control group and the 5-min SKF group ($p < 0.05$). Representative blots are shown.

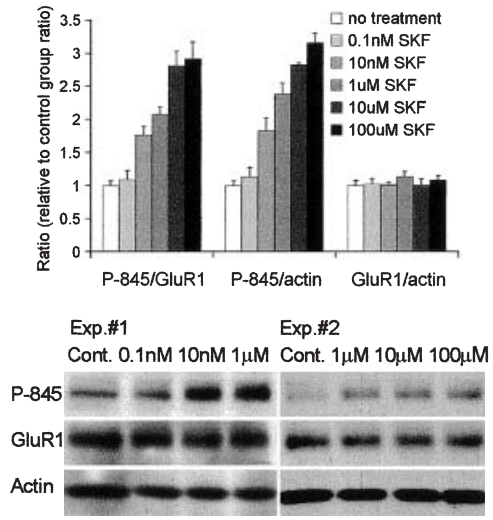

Fig. 2 The D_1 receptor agonist SKF 81297 produced a concentration-dependent increase in GluR1 phosphorylation at the PKA site (Ser845). SKF 81297 (0.1 nM, 10 nM, 1 μM, 10 μM, or 100 μM) was added to cultures for 15 min. The ratios of P-845/total GluR1, P-845/actin and total GluR1/actin were determined for each sample and normalized to the control group ratio. Triplicates were run for each condition. Data are plotted as the mean ± SEM. Analysis of P-845/GluR1 data using a Kruskal–Wallis one-way ANOVA on ranks indicated a significant effect of concentration ($F_{5,18} = 31.0$, $p < 0.001$). *Post-hoc* analysis indicated that all concentrations of SKF 81297 except 0.1 nM were significantly different from the control (Dunnett's test, $p < 0.05$). Representative blots are shown.

determined with a phosphorylation-independent antibody. In all experiments, triplicates of each condition were loaded onto the same gel for direct comparison. To enable comparison of different blots with variable exposure times, results from experimental groups were normalized to the mean of control lanes from the same gel.

In the first experiment, shown in Fig. 1, cultures were incubated with 1 μM SKF 81297 for different lengths of time (5–60 min). Results were expressed as the ratio of GluR1 phosphorylated at Ser845 to total GluR1 (P-845/GluR1). This ratio increased to 183 ± 25% of control levels within 5 min ($p < 0.05$ compared with control) and remained at approximately this level for 60 min. The ratio of total GluR1 to actin remained constant throughout the experiment, verifying that SKF 81297 did not influence total levels of GluR1 protein in the cultures.

In the second experiment, increasing concentrations of SKF 81297 (0.1 nM to 100 μM) were applied to cultures for 15 min (Fig. 2). An equivalent amount of vehicle (water) was added to control wells. At concentrations as low as 10 nM, SKF 81297 produced a marked increase in GluR1 Ser845 phosphorylation (176 ± 13% of control; $p < 0.05$ compared with control). Phosphorylation was maximal at 10 μM SKF-81297 (281 ± 22% of control), as a 10-fold higher concentration of SKF 81297 (100 μM) had no further effect (291 ± 26% of control). The ratio of total GluR1 to actin did not change with any concentration of SKF 81297 tested.

Pharmacology of DA receptor-mediated regulation of GluR1 phosphorylation

To determine if the effect of SFK 81297 was mediated by D_1 receptors, experiments were performed with the D_1 receptor antagonist SCH 23390. As shown in Fig. 3, incubation with 1 μM SKF 81297 for 15 min produced a marked increase (188 ± 10%; $p < 0.05$ compared with control) in GluR1 Ser845 phosphorylation. This effect was completely blocked by pre-treating the cultures for 5 min with SCH 23390 (99 ± 8% of control; $p < 0.05$ compared with SKF 81297 alone). SCH 23390 alone did not produce any effect on GluR1 Ser845 phosphorylation (104 ± 6% of control). As a positive control for phosphorylation of GluR1 at the PKA site (Ser845), we used the adenylyl cyclase activator

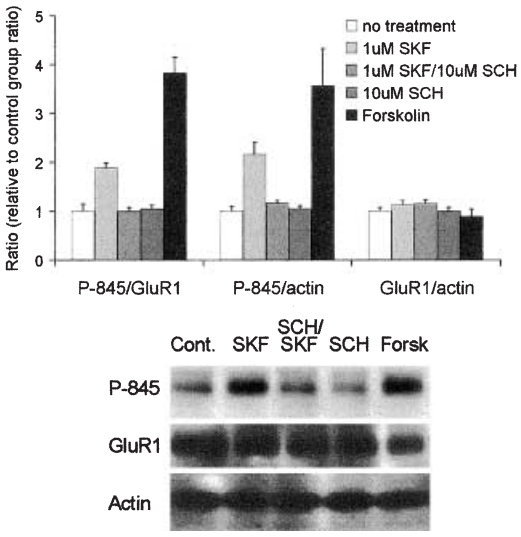

Fig. 3 The D_1 receptor antagonist SCH 23390 reversed the increase in GluR1 Ser845 phosphorylation produced by the D_1 agonist SKF 81297. Cultures were incubated with 1 μM SKF 81297 for 15 min, either with or without a 5-min pre-treatment with 10 μM SCH 23390. Forskolin (10 μM in DMSO) was used as a positive control. The ratios of P-845/total GluR1, P-845/actin and total GluR1/actin were determined for each sample and normalized to the control group ratio. Samples were run in triplicate (or greater) for each condition. Data are plotted as the mean ± SEM. Analysis of P-845/GluR1 data using a Kruskal–Wallis one-way ANOVA on ranks indicated significant group differences ($F_{3,8} = 19.28$, $p < 0.001$). The SKF and forskolin groups differed significantly from the control group (Tukey test; $p < 0.05$). In addition, the SKF group differed significantly from the SKF/SCH group and from the SCH-only group (Tukey test; $p < 0.05$). Representative blots are shown.

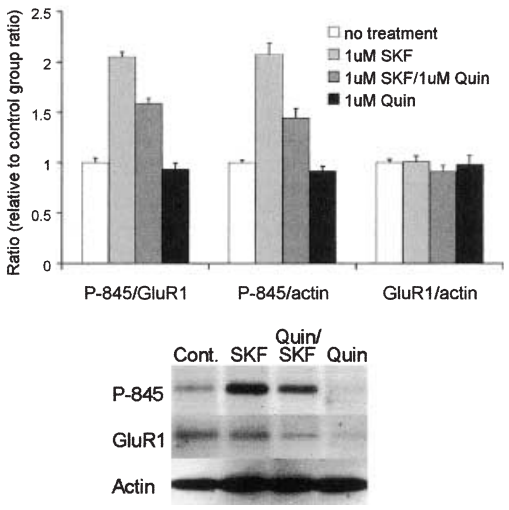

Fig. 4 The D_2 receptor agonist quinpirole (1 μM) attenuated the increase in GluR1 phosphorylation produced by the D_1 agonist SKF 81297. Cultures were incubated with 1 μM SKF 81297 for 15 min, either with or without a 5-min pre-incubation with quinpirole. The ratios of P-845/total GluR1, P-845/actin and total GluR1/actin were determined for each sample and normalized to the control group ratio. Triplicates were run for each condition. Data are plotted as the mean ± SEM. Analysis of P-845/GluR1 data using a Kruskal–Wallis one-way ANOVA on ranks indicated significant group differences ($F_{3,8} = 110.0$, $p < 0.001$). The SKF and SKF/Quin groups differed significantly from the control group, and the SKF/Quin group differed significantly from the SKF group (Tukey test; $p < 0.05$). Representative blots are shown.

forskolin (10 μM). Forskolin produced more than a threefold increase in GluR1 Ser845 phosphorylation, regardless of whether results were expressed as P-845/GluR1 (383 ± 32% of control) or P-845/actin (356 ± 76% of control), and had no effect on total GluR1 levels in the membrane fraction compared with actin (89 ± 15% of control).

The D_2 receptor agonist quinpirole was also applied to the cultures to study its effect on GluR1 phosphorylation (Fig. 4). With 1 μM quinpirole alone, phosphorylation of GluR1 at Ser845 was not changed significantly (94 ± 6% of control). However, when applied with SKF 81297, quinpirole significantly attenuated the effect of SKF 81297 on GluR1 Ser845 phosphorylation (158 ± 5% of control compared with 205 ± 5% with SKF alone; $p < 0.05$).

D_1 receptors modulate GluR1 phosphorylation at the PKA site

To verify that the D_1 receptor-mediated increase in GluR1 phosphorylation involves the PKA pathway, the PKA inhibitor H89 was applied to cultures. As shown in Fig. 5, pre-treatment with 20 μM H89 for 5 min completely blocked the effect of SKF 81297 on GluR1 Ser845 phosphorylation (200 ± 18% of control for SKF alone vs. 58 ± 3% for SKF/H89; $p < 0.05$). We also examined whether DA receptors influence phosphorylation of GluR1 at the site phosphorylated by calcium/calmodulin-dependent protein kinase II and protein kinase C using a Ser831 phosphorylation site-specific antibody (Mammen *et al.* 1997). We found no significant changes in the ratio of GluR1 phosphorylated at Ser831 to total GluR1 (P-831/GluR1) after 30 min of incubation with SKF 81297 (10 μM; 102.4 ± 3.2% of control), quinpirole (10 μM; 95.4 ± 3.9% of control), SCH 23390 (10 μM; 90.3 ± 5.6% of control), or the D_2 receptor antagonist eticlopride (10 μM; 83.5 ± 4.4% of control).

Discussion

Our results show that GluR1 is phosphorylated at the PKA site by stimulation of D_1 receptors in nucleus accumbens neurons. The response is robust (twofold maximal increase), sensitive (evident at 10 nM SKF 81297), and D_1 DA receptor-specific (blocked by SCH 23390). It is selective for the PKA site, because no changes are observed at the site phosphorylated by protein kinase C and calcium/

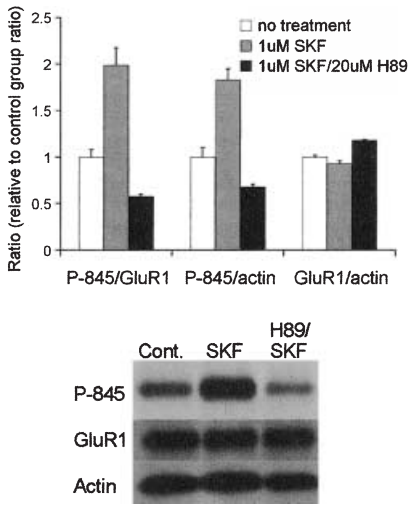

Fig. 5 The PKA inhibitor H89 inhibited basal and D_1 receptor-stimulated phosphorylation of GluR1. H89 (20 μM) was added to cultures with or without SKF 81297. The ratios of P-845/total GluR1, P-845/actin and total GluR1/actin were determined for each sample and normalized to the control group ratio. Triplicates were run for each condition. Data are plotted as the mean ± SEM. Analysis of P-845/GluR1 data using a Kruskal–Wallis one-way ANOVA on ranks indicated significant group differences ($F_{2,6} = 35.58$, $p < 0.001$). The SKF and SKF/H89 groups differed significantly from the control group, and the SKF/H89 group differed significantly from the SKF group (Tukey test; $p < 0.05$). Representative blots are shown.

calmodulin-dependent protein kinase II (Ser831). In other systems, phosphorylation of GluR1 at the PKA site has been shown to enhance AMPA receptor currents (see Introduction). Thus, our results suggest a potential mechanism to account for synergistic interactions between DA and AMPA receptors in the nucleus accumbens.

DA and AMPA receptors in nucleus accumbens primary cultures

In characterizing our system, we performed double immunofluorescence staining studies on fixed cultures (Chao *et al.* 1999). First, we showed that the vast majority of neurons (identified with βIII tubulin) express glutamic acid decarboxylase, in agreement with work showing that medium spiny GABAergic neurons comprise 90% of the neurons in adult rat nucleus accumbens (Meredith and Totterdell 1999) and nucleus accumbens cultures (Shi and Rayport 1994). Second, double staining for GluR1 and βIII tubulin indicated that GluR1 is present in nearly all neurons, consistent with *in vivo* results (Chen *et al.* 1998). Third, double staining for D_1 or D_2 receptors and βIII tubulin indicated the presence of each DA receptor subtype on 80% of our cultured neurons, again consistent with published findings (Rayport and Sulzer 1995; Shetreat *et al.* 1996). In summary, almost all neurons in the cultures are GABAergic medium spiny neurons expressing GluR1, and many also express D_1 or D_2 receptors. Thus, our results probably reflect interactions between D_1 receptors and GluR1 at the single cell level.

D_1 receptor-mediated potentiation of AMPA receptor currents

Prior studies in hippocampal neurons, cortical neurons and transfected cells have shown that activation of PKA increases AMPA receptor currents (Greengard *et al.* 1991; Wang *et al.* 1991; Blackstone *et al.* 1994; Rosenmund *et al.* 1994; Roche *et al.* 1996). In studies of the dorsal striatum, D_1 receptor stimulation enhanced AMPA receptor currents through a PKA-dependent mechanism in acutely dissociated neurons (Yan *et al.* 1999) and cultured embryonic neurons (Price *et al.* 1999). The mechanism involves an increase in peak open probability of the AMPA receptor channel (Banke *et al.* 2000). A study in mouse striatal slices showed that D_1 receptor stimulation increased GluR1 phosphorylation at Ser845 and that *in vivo* treatment with cocaine or methamphetamine similarly increased GluR1 phosphorylation, but AMPA receptor currents were not examined (Snyder *et al.* 2000). The present study is the first to demonstrate that D_1 receptors enhance GluR1 phosphorylation in the nucleus accumbens, and includes a more thorough analysis of concentration-dependence and time–course for D_1 receptor-mediated effects on GluR1 phosphorylation. Our study is also the first to report that D_2 receptor agonists attenuate the increase in GluR1 phosphorylation produced by D_1 receptor agonists. This is consistent with opposing effects of D_1 and D_2 receptors on PKA (Stoof and Kebabian 1981). The only prior study to examine this issue found no effect of D_2 receptor stimulation on GluR1 phosphorylation at Ser845 (Snyder *et al.* 2000). The latter study was performed in striatal slices, where trans-synaptic effects of D_2 receptor activation could have obscured direct interactions of D_1 and D_2 receptors at the level of PKA.

Potential mechanisms for regulation of AMPA receptors by D_1 receptors

The simplest explanation for our results is that D_1 receptor stimulation activates PKA, thereby increasing phosphorylation of GluR1. However, another possibility is that PKA activation leads to phosphorylation of DARPP-32, interfering with dephosphorylation of GluR1. The latter pathway contributes to DA- and PKA-mediated increases in phosphorylation of GluR1 (Snyder *et al.* 2000) and the NMDA receptor subunit NR1 (Blank *et al.* 1997; Snyder *et al.* 1998). It is also implicated in long-term potentiation (LTP) and long-term depression (LTD) in the corticostriatal pathway (Calabresi *et al.* 2000).

In other experiments using postnatal nucleus accumbens cultures, we have found that D_1 receptor stimulation increases the surface expression of GluR1 both in medium

spiny neurons and interneurons, an effect that is reproduced by the adenylyl cyclase activator forskolin (Chao *et al*. 2000). Results in other systems support a relationship between PKA activity and AMPA receptor reinsertion into the membrane during recycling (Ehlers 2000). Together, these findings suggest that D_1 receptor stimulation increases GluR1 surface expression by promoting AMPA receptor recycling through a PKA-dependent mechanism. However, many additional studies are necessary to define the relationship between D_1 receptor-mediated effects on GluR1 phosphorylation and GluR1 surface expression in the nucleus accumbens.

Functional significance of D_1 and AMPA receptor interactions

Interactions between DA and glutamate receptors in the striatal complex have been extensively studied by electrophysiological methods. Both inhibitory and excitatory effects of DA on glutamate-evoked activity have been reported depending on the recording preparation, the concentration of DA, and the subtypes of glutamate and DA receptors activated (Chiodo and Berger 1986; Kiyatkin and Rebec 1996; Hu and White 1997; Cepeda and Levine 1998). For example, while many studies using microiontophoretic techniques have reported inhibitory effects of D_1 receptor agonists on the glutamate-evoked activity of striatal or nucleus accumbens neurons (e.g. White and Wang 1986; Henry and White 1991), DA or D_1 agonists applied at low ejection currents will facilitate excitatory effects of glutamate in anesthetized rats (Chiodo and Berger 1986; Hu and White 1997) and in freely moving rats (Kiyatkin and Rebec 1996). The membrane potential of the striatal neuron, set by the level of afferent drive, is also a critical factor in determining the direction of the response to D_1 receptor stimulation (reviewed by Nicola *et al*. 2000). Medium spiny neurons show membrane potential shifts, between a hyperpolarized 'down state' and a depolarized 'up state', that are important for information processing in the NAc (Wilson 1993; O'Donnell and Grace 1995; Goto and O'Donnell 2001). Studies in striatal slices have shown that D_1 receptor activation increases spike activity when recordings are performed at depolarized membrane potentials (Hernandez-Lopez *et al*. 1997) but inhibits spike activity at hyperpolarized membrane potentials (Calabresi *et al*. 1987; Hernandez-Lopez *et al*. 1997).

Recent studies performed *in vivo* argue for the importance of D_1 receptor-mediated excitatory effects in the intact striatum and nucleus accumbens. Gonon and Sundstrom (1996) showed that endogenous DA, released in a physiological manner by enhancing impulse flow in the meso-accumbens DA pathway, excited the majority of nucleus accumbens neurons analyzed via a D_1 receptor-dependent mechanism and also enhanced excitatory responses produced by stimulation of hippocampal projections to the nucleus accumbens. Similar results were found in dorsal striatum (Gonon 1997). D_1 receptors also potentiate basolateral amygdala-evoked firing of nucleus accumbens neurons (Floresco *et al*. 2001). In a very recent study, West and Grace (2002) applied a D_1 receptor antagonist by reverse dialysis while performing *in vivo* intracellular recordings from striatal neurons, and observed a decrease in the maximal depolarized membrane potential, a decrease in the amplitude of up-state events, and an increase in the intracellular current injection amplitude required to elicit an action potential. These findings suggest that D_1 receptor stimulation in the intact striatum exerts a tonic excitatory influence on medium spiny neurons.

While some excitatory effects of D_1 receptors on nucleus accumbens neurons involve potentiation of NMDA rather than AMPA receptor-mediated responses (Floresco *et al*. 2001), AMPA receptors mediate most excitatory transmission in the nucleus accumbens (Pennartz *et al*. 1990, 1991; Hu and White 1996). Thus, our results suggest a cellular mechanism that may contribute to many of the observed synergistic interactions between D_1 and excitatory transmission, that is, D_1 receptors enhance AMPA receptor currents by stimulating phosphorylation of GluR1 at the PKA site. This hypothesis is consistent with results showing that PKA activators and inhibitors enhance and depress, respectively, excitatory postsynaptic potentials evoked by electrical stimulation of striatal slices (Colwell and Levine 1995). Activation of L-type calcium channels may also be involved in D_1 receptor modulation of AMPA receptor currents (Galarraga *et al*. 1997; Hernandez-Lopez *et al*. 1997; Cooper and White 2000).

Stimulant-induced neuroadaptations in nucleus accumbens neurons

While the present experiments focused on acute DA/glutamate receptor interactions, a major reason for undertaking this study was to identify mechanisms that might account for plasticity in nucleus accumbens neurons. Cocaine and amphetamine initially target the DA transporter, leading to an acute increase in synaptic DA levels. However, chronic drug administration produces changes in glutamate transmission that are critical for neuroadaptations related to addiction (Wolf 1998, 2001). For example, repeated amphetamine administration decreases AMPA receptor subunit expression (Lu *et al*. 1997; Lu and Wolf 1999) and electrophysiological responses to glutamate (White *et al*. 1995) in the nucleus accumbens. Recent studies show that behaviorally sensitizing regimens of cocaine produce LTD-like effects in the nucleus accumbens (Thomas *et al*. 2001) and LTP-like effects in the ventral tegmental area (Ungless *et al*. 2001).

Very little is known about mechanisms by which chronic over-stimulation of DA receptors, as a result of repeated psychostimulant administration, ultimately leads to

alterations in glutamate transmission. A logical starting point is to identify ways in which acute DA receptor stimulation can influence glutamate receptors. The present results show that D_1 receptor stimulation increases GluR1 phosphorylation in the nucleus accumbens through a PKA-dependent mechanism. As noted above, we have found that D_1 receptor stimulation increases GluR1 surface expression in the same culture system (Chao *et al*. 2000). Alterations in AMPA receptor subunit phosphorylation and synaptic localization are critical to LTP and LTD. For example, phosphorylation and dephosphorylation of GluR1 at both Ser845 and Ser831 may contribute to LTP and LTD in hippocampal neurons (Lee *et al*. 2000), while AMPA receptor surface expression is increased in LTP and decreased in LTD (reviewed by Malinow *et al*. 2000; Carroll *et al*. 2001). By modulating AMPA receptor phosphorylation and synaptic localization, D_1 receptors may influence plasticity in the striatal complex.

After chronic stimulant administration, both D_1 receptor function and the cAMP-PKA signaling cascade show profound adaptations (Self and Nestler 1995; White and Kalivas 1998). This may influence D_1 receptor-mediated regulation of AMPA receptors. Indeed, a recent study in dorsal striatum found reduced phosphorylation of GluR1 at the PKA site and reduced peak amplitudes of AMPA/kainate-evoked currents after repeated treatment with cocaine (Bibb *et al*. 2001). Our findings predict that drug-induced adaptations in D_1 receptor signaling will alter AMPA receptor phosphorylation and synaptic localization in the nucleus accumbens. This could play an important role in producing the maladaptive forms of plasticity that contribute to drug addiction.

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