Published in final edited form as:

Synapse. 2009 October ; 63(10): 863–870. doi:10.1002/syn.20669.

Regulation of dynamin 2 and G protein-coupled receptor kinase 2 in rat nucleus accumbens during acute and repeated cocaine administration

Joseph A. Schroeder^{1,3}, Mary R. McCafferty¹, and Ellen M. Unterwald^{1,2}

¹Temple University School of Medicine, Dept of Pharmacology and the Center for Substance Abuse Research, Philadelphia, PA 19140

²The Rockefeller University, Laboratory of the Biology of Addictive Diseases, New York, NY 10021

Abstract

Exposure to cocaine causes many neuroadaptations including alterations in several neurotransmitter receptors and transporters. This study investigated potential mechanisms of cocaine-induced receptor and transporter regulation by measuring levels of two proteins involved in receptor and transporter trafficking, dynamin 2 and G protein-coupled receptor kinase 2 (GRK2). Male Fischer rats received three daily injections of cocaine, 15 mg/kg, in a binge-pattern (at one hour intervals) for 1, 3, or 14 days. Brain regions of interest were collected 30 minutes after the last injection and proteins measured by Western blot. Acute binge-pattern cocaine administration produced a significant increase in both dynamin 2- and GRK2-immunoreactivity (227% and 358% of control) in the nucleus accumbens and GRK2 (150% of control) in the caudate putamen. Tolerance to this effect occurred, as levels of both proteins returned to baseline after 3 days of cocaine. In contrast, dynamin 2 and GRK2 were significantly decreased in the nucleus accumbens after chronic cocaine. This pattern of regulation was unique to the nucleus accumbens and not seen in the frontal cortex or substantia nigra. Pre-treatment with either the dopamine D1 receptor antagonist SCH 23390 or D2 receptor antagonist eticlopride prior to acute cocaine blocked the upregulation of dynamin 2 and GRK2 in the nucleus accumbens. However, only eticlopride was effective in attenuating the decrease in these proteins following chronic cocaine exposure. These results demonstrate that two proteins involved in receptor and transporter trafficking are selectively regulated in the nucleus accumbens following acute versus chronic cocaine exposure, and dopamine receptor activation is required for this regulation.

Keywords

GRK2; striatum; binge cocaine; dynamin 2; receptor trafficking

INTRODUCTION

Increases in synaptic dopamine concentrations resulting from direct inhibition of the dopamine transporter (DAT) by cocaine have profound effects on the neurochemistry of dopaminergic synapses and account for a variety of cocaine-induced behaviors. Both acute and chronic cocaine administration have been shown to alter the levels and/or activity of

Corresponding author: Ellen M. Unterwald, Ph.D. Department of Pharmacology Temple University School of Medicine 3420 N Broad St. MRB321 Philadelphia, PA 19140 ph: (215)707-1681 fax: (215) 707-7068 ellen.unterwald@temple.edu.

³current address: Department of Psychology, Connecticut College, New London, CT

DAT, however the results of these studies are conflicted with reported of increases, decreases, or no changes in DAT appearing in the literature (Cass et al., 1993; Pilotte et al., 1994; Zahniser and Doolen, 2001; Chefer and Shippenberg, 2002; Izenwasser, 2004). Likewise, numerous studies have examined the effects of acute and/or chronic cocaine administration on dopamine D1 and D2 receptor levels with inconsistent findings (Goeders and Kuhar, 1987; Kleven et al., 1990; Peris et al., 1990; Unterwald et al., 1994). Reports of increases, decreases or no changes in striatal DAT and dopamine receptor levels in response to cocaine suggest that the dynamics of synaptic protein expression is a complex, plastic process that is mediated by a number of interconnected factors. Our previous results indicate that the frequency, duration and pattern of cocaine administration are critical factors affecting the cellular responses to repeated cocaine administration (Unterwald et al., 2001). Likewise, contingent versus non-contingent cocaine administration can produce different neurochemical and behavioral adaptations (Lecca et al., 2007; Miguens et al., 2008). An understanding of the role of synaptic protein trafficking machinery in shaping the synaptic landscape is important for identifying neurochemical mechanisms responsible for psychostimulant-related behaviors and neuroplasticity.

The dynaminins are a class of GTPase mechanoenzymes that are centrally involved in endocytosis and receptor trafficking (reviewed in Dannino and Hinshaw, 2001). Dynamin 2 is a ubiquitously-expressed isoform (Cook et al., 1994) that has been shown to regulate the agonist-dependent internalization of dopamine D2 (Iwata et al., 1999; Kabbani et al., 2004) and D1 receptors (Zhang et al., 2007), as well as DAT (Daniels and Amara, 1999; Saunders et al., 2000). Another family of receptor trafficking proteins, G-protein-receptor kinases (GRKs), phosphorylate G-protein coupled receptors leading to arrestin-mediated uncoupling of the receptor from the G-protein complex and subsequent desensitization (reviewed in Ferguson et al., 1996). The GRK2 isoform is widely expressed throughout the brain (Erdtmann-Vourliotis et al., 2001). GRK2 has been shown to be involved in both D1 and D2 receptor internalization (Tiberi et al., 1996; Ito et al., 1999; Lamey et al., 2002) and D2 receptor sequestration may be co-dependent on dynamin and GRK2 (Iwata et al., 1999). GRK2 and dynamin 2 also regulate the internalization of other receptors altered by cocaine exposure, including mu opioid receptors (Unterwald et al., 1992; Keith et al., 1998). The response of these membrane protein sequestration mechanisms is rapid (Vickery and von Zastrow, 1999) and they likely serve as a reactive and efficient mechanism for regulating synaptic proteins and neurotransmitter responses.

In vitro studies have significantly aided our understanding of the complexities of synaptic membrane protein trafficking processes, however investigations focused on how membrane protein sequestration machinery is affected by psychostimulants *in vivo* are lacking. Saunders et al. (2000) demonstrated that the efficiency of synaptic dopamine clearance in response to amphetamine in human embryonic kidney (HEK) cells is reduced by dynamin-mediated internalization of DAT. While the results from HEK cells are intriguing, questions concerning the regulation of proteins critical to the trafficking process during *in vivo* exposure to cocaine have largely gone unanswered. It is possible that cocaine-induced alterations in membrane-bound proteins are mediated by an up- or down-regulation of the internalization machinery. This regulation may change as a function of the length and pattern of cocaine administration. In addition, given the profound effect that cocaine has on synaptic dopamine concentrations, it is likely that any changes in dynamin and GRK2 expression might be mediated by dopamine receptor activation. The data presented here are the result of two investigations. The first study sought to determine if the levels of the receptor trafficking proteins dynamin 2 and GRK2 are altered in specific regions of rat brain following acute and repeated administration of cocaine. The finding that both dynamin 2 and GRK2 are regulated differentially in response to acute versus chronic cocaine initiated a

follow-up investigation of the role of D1 and D2 receptors in cocaine-induced dynamin 2 and GRK2 regulation.

MATERIALS AND METHODS

Animals and drug injections

Young adult (approximately 60 days old) male Fischer rats obtained from Charles River Laboratories (Raleigh, NC) were used for both studies. Animals were group-housed, maintained on a 12-h light/dark cycle (7:00 AM to 7:00 PM) and received food and water *ad libitum*. All animal procedures were consistent with the NIH Guide for Care and Use of Laboratory Animals (NIH Publication #80-23) and were approved by the Temple University Institutional Animal Care and Use Committee.

Cocaine hydrochloride (generously provided by NIDA) was dissolved in sterile saline and a dose of 15 mg/kg was injected intraperitoneally (ip) three times daily at one-hour intervals (ie, binge-pattern) at 9:00, 10:00, 11:00 AM for 1, 3 or 14 days (Unterwald et al., 2001). Control animals were injected with an equivalent volume of saline (1 ml/kg body weight) using the same injection schedule. For the second study, animals were pretreated 30 minutes prior to the first daily cocaine or saline injection with either the dopamine D1 receptor antagonist, SCH 23390 (0.5 mg/kg ip), the selective D2 receptor antagonist, eticlopride (1 mg/kg ip), or saline (1 ml/kg ip). Thirty minutes later, animals received three injections of saline or cocaine (15 mg/kg ip) in a binge-pattern and this procedure was carried out for 1 or 14 days.

Activity measurement

Activity was measured using a Digiscan D Micro System (Accuscan, Columbus, OH). Each activity monitor consists of an aluminum frame equipped with 16 infrared light beams and detectors into which a standard plastic rat cage is placed. As the animal moves about the chamber, the beams are broken and recorded by a computer interfaced to the monitors. Animals were placed into the monitors and ten minutes later, they were pretreated with SCH23390, eticlopride, or saline and returned to the monitors. Binge-pattern cocaine or saline administration began thirty minutes after the pretreatment whereby cocaine or saline was administered three times at one hour intervals and activity was measured for 180 minutes. Means $\pm$ SEM activity counts for each treatment group were calculated for each 10 minute period during the session. Statistical significance was determined using an analysis of variance (ANOVA). Bonferroni's Multiple Comparison Test was performed with a significant ANOVA.

Western Blots

Animals were euthanized 30 minutes after the last cocaine or saline injection. Specific brain regions were taken by rapid gross dissection on ice, and included the nucleus accumbens, caudate putamen, frontal cortex, and substantia nigra. Tissues from individual animals were sonicated in 1% SDS boiling buffer, boiled for 5 min, aliquoted and stored at -80°C until assayed. Protein determinations were made using the Lowry method (Lowry et al., 1951). Protein extracts (10 μg protein) were subjected to SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. Membranes were blocked for 1-h in blocking buffer, containing 5% nonfat dry milk and Tween-TBS and then incubated with antibodies to dynamin 2 and GRK2 (Santa Cruz Biotechnology). After washing, the membranes were incubated in the appropriate secondary antibody conjugated to horseradish peroxidase (Vector Laboratories) for 1-h. Immunoreactivity was visualized by chemiluminescence and quantified using densitometry. All blots were probed for tubulin as a control for the amount of protein loaded on the gel and transfer efficiency. Data were analyzed as a ratio of protein

of interest to tubulin. Statistical significance was determined using an un-paired t-tailed t-test or one-way ANOVA followed by Bonferroni's Multiple Comparison Test with a significant ANOVA. The null hypothesis was rejected when $P < 0.05$.

RESULTS

The objective of the first study was to evaluate the impact of acute and repeated administration of cocaine on the levels of dynamin 2 and GRK2 in the nucleus accumbens, caudate putamen, frontal cortex and substantia nigra. Levels of dynamin 2 and GRK2 in each brain region are displayed in Figure 1 and expressed as a percent of the levels in control animals injected with saline for an equal number of days. Dynamin 2 levels in the nucleus accumbens (Fig. 1A) were significantly higher following acute binge-pattern cocaine administration (+227% of control) as compared to saline matched controls ($t=6.08$, $df=10$, $p<0.0001$). The levels of dynamin 2 in the accumbens were not significantly altered following 3 days of binge-pattern cocaine administration, but were significantly decreased (−33%) after 14 days of cocaine exposure ($t=2.39$, $df=11$, $p < 0.035$). This differential regulation of dynamin 2 expression in response to acute versus chronic cocaine exposure was mirrored by the regulation of GRK2. GRK2 levels were significantly elevated (+358% of control) in response to 1 day of binge-pattern cocaine administration ($t=6.21$, $df=11$, $p<0.0001$) and significantly reduced (−35%) following 14 days of cocaine ($t=3.77$, $df=11$, $p=0.003$). Representative immunoblots of dynamin 2 and GRK2 in the accumbens are shown in Figure 2. In the caudate putamen (Fig. 1B), GRK2 levels were significantly elevated after one day of binge-pattern cocaine ($t=2.44$, $df=11$, $p=0.03$). Dynamin 2 and GRK2 levels were not altered after 3 or 14 days of cocaine in the caudate putamen. In the frontal cortex (Fig. 1C), dynamin 2 was significantly reduced following one day of cocaine as compared to saline-injected controls ($t=3.58$, $df=13$, $p=0.003$). No other changes in dynamin 2 or GRK2 were noted in this brain region. No changes in dynamin 2 or GRK2 levels were observed in the substantia nigra following 1, 3 or 14 days of binge-pattern cocaine administration (Fig. 1D). These results indicate that acute cocaine produces a rapid upregulation of these trafficking proteins selectively in the striatum, while chronic administration causes a down-regulation of the same in the nucleus accumbens.

The second study investigated if the changes in dynamin 2 and GRK2 levels in the nucleus accumbens in response to cocaine were mediated by dopamine D1 and/or D2 receptors. The D1 receptor antagonist SCH23390 (0.5 mg/kg) or the D2 receptor antagonist eticlopride (1.0 mg/kg) was administered 30 minutes prior to daily cocaine exposure. As shown in Figure 3, the increases in dynamin 2 and GRK2 levels in the accumbens observed in response to acute (1 day) cocaine exposure were attenuated by pre-treatment with either SCH23390 or eticlopride. An ANOVA showed a significant difference between groups in levels of dynamin 2 ($F[5, 26] = 5.63$, $p = 0.0012$). Bonferroni's Multiple Comparison Test indicates that dynamin 2 abundance in the saline + cocaine group was significantly different than that in the SCH23390 + cocaine ($p<0.01$) or eticlopride + cocaine ($p<0.001$) groups. Likewise, ANOVA revealed a significant difference between GRK2 levels ($F[5,29] = 22.08$, $p < 0.0001$). Post-test showed significant differences between saline + cocaine versus SCH23390 + cocaine ($p<0.001$) or eticlopride + cocaine ($p<0.001$). SCH23390 or eticlopride alone were not significantly different than the saline + saline control group for either dynamin 2 or GRK2 levels ($p>0.05$; Figure 3).

Chronic 14-day binge-pattern cocaine administration resulted in a down-regulation of dynamin 2 and GRK2 in the nucleus accumbens. Pretreatment with eticlopride, but not SCH23390, prior to daily cocaine for 14 days prevented this down-regulation (Figure 4). ANOVA showed a significant difference between treatment groups for dynamin 2 ($F[5,36] = 4.9$, $p = 0.0016$). Bonferroni's Multiple Comparison Test demonstrates that the abundance

of dynamin 2 in the saline + cocaine group was not significantly different from that in the SCH23390 + cocaine group ($p > 0.05$), but was significantly less than that in animals administered eticlopride + cocaine ($p < 0.05$). Likewise, only eticlopride pre-treatment was effective in attenuating the reduction in accumbens GRK2 levels produced by chronic cocaine exposure ($F [5, 40] = 4.56, p = 0.0022$). Post-hoc test show a significant difference in GRK2 levels between the saline + cocaine group and the eticlopride + cocaine group ($p < 0.01$), but not the SCH23390 + cocaine group ($p > 0.05$). Dynamin 2 and GRK2 levels were not significantly altered by SCH23390 or eticlopride alone (all p 's > 0.05).

Locomotor activity was measured in the animals from the second study in order to determine if the doses of SCH23390 and eticlopride used were sufficient to block cocaine-induced hyperactivity. ANOVA of the activity data from the first day of drug administration showed a significant difference between treatment groups ($F[2,51] = 145.1, p < 0.0001$). Bonferroni's Multiple Comparison Test demonstrated that both SCH23390 ($p < 0.001$) and eticlopride ($p < 0.001$) were effective in blocking cocaine-induced hyperactivity (Figure 5 left). SCH23390 + cocaine and eticlopride + cocaine groups were not significantly different from each other or from saline + saline controls ($p > 0.05$; saline data alone not shown). Activity was measured every other day during the study and results showed that pretreatment with SCH23390 or eticlopride significantly blocked cocaine-induced activity on each day (days 3, 5, 7, 9, 11, and 13 of the study), similar to what was found on day 1. Activity measured on day 13 of drug administration is shown in Figure 5 (right). A significant difference between treatment groups was found ($F[2,51] = 150.1, p < 0.0001$). Bonferroni's Multiple Comparison Test demonstrated that both SCH23390 ($p < 0.001$) and eticlopride ($p < 0.001$) blocked cocaine-induced hyperactivity on day 13, similar to what was found on day 1.

DISCUSSION

The data presented here demonstrate that the receptor trafficking proteins, dynamin 2 and GRK2, are regulated differentially by acute versus chronic cocaine administration in the nucleus accumbens of adult male rats. One possible cellular response to cocaine-induced increases in extracellular dopamine concentrations and presumably excessive dopamine receptor activation, may be the internalization of dopamine D1 receptors, D2 receptors and/or DAT. *In vitro* studies have demonstrated that the internalization of D1 and D2 receptors following their activation is rapid and may involve both dynamin-dependent and independent mechanisms (Vickery and von Zastrow, 1999). Our data support this by indicating that acute cocaine administration is accompanied by a dramatic increase in the levels of two receptor trafficking proteins that are involved in internalization processes. The rapid upregulation of both dynamin 2 and GRK2 was prevented by co-administration of SCH23390 and eticlopride implying that the response is controlled by activation of D1 and D2 receptors. The upregulation of dynamin 2 and GRK2 might mediate the rapid internalization of dopamine receptors or DAT in an attempt to normalize dopamine signaling in response to high levels of synaptic dopamine resulting from cocaine. The normalization of dynamin 2 and GRK2 levels that was seen following three days of repeated cocaine indicates that synaptic protein trafficking, including the expression of the trafficking proteins themselves, is a dynamic and tightly controlled process. This dynamic regulation of internalization machinery may help explain the inconsistencies in the literature on the regulation of dopamine receptors (Goeders and Kuhar, 1987; Kleven et al., 1990; Peris et al., 1990; Unterwald et al., 1994) and DAT (Cass et al., 1993; Pilotte et al., 1994; Zahniser and Doolen, 2001; Chefer and Shippenberg, 2002; Izenwasser, 2004) during cocaine administration.

Down-regulation of dynamin 2 and GRK2 protein in the nucleus accumbens was found following two weeks of binge-pattern cocaine administration, in contrast to the upregulation that was seen after acute cocaine exposure. This suggests that the response of membrane trafficking processes to repeated blockade of dopamine uptake is biphasic and may be reflective of physiologic changes at the synaptic level that contribute to the development of cocaine sensitization. The binge-pattern cocaine administration paradigm is designed to better mimic the pattern of drug abuse in humans, although it is appreciated that non-contingent drug administration may produce effects not entirely consistent with those produced by contingent drug administration procedures (Lecca et al., 2007; Miguens et al., 2008). Previous data indicate that chronic binge-pattern cocaine administration produces an upregulation of D1 receptors in the nucleus accumbens (Unterwald et al., 1994, 2001), and perhaps D1 receptor upregulation is a result of the decrease in trafficking proteins noted here.

Cocaine administered in a binge-pattern produced the expected increase in locomotor activity, and the magnitude of the response was larger on day 13 than on day 1 indicating the development of behavioral sensitization. This result is similar to what we have previously reported following chronic binge-pattern cocaine administration (Unterwald et al., 1994). Pretreatment with either the D1 receptor antagonist SCH23390 or the D2 receptor antagonist eticlopride prevented cocaine-induced hyperactivity on all days of the study. Likewise, both SCH23390 and eticlopride attenuated the dramatic upregulation of dynamin 2 and GRK2 produced by acute cocaine administration. Therefore the doses of the antagonists chosen were sufficient to attenuate cocaine-induced hyperactivity and neurochemical changes.

The ability of eticlopride but not SCH23390 to block cocaine-induced down-regulation of dynamin 2 and GRK2 in the nucleus accumbens following chronic exposure to cocaine demonstrates that D2 receptor activation is critically involved in the regulation of these trafficking proteins under conditions of chronic cocaine administration. This could reflect an increase in D2 receptor levels that has been reported following chronic cocaine administration (Kleven et al., 1990), but also could be due to D2 receptor-mediated effects on the expression of other neurotransmitter receptors. For example, mu opioid receptors in the accumbens are upregulated following chronic cocaine administration (Hammer, 1989; Unterwald et al., 1992), by a mechanism that likely involves D2 receptors (Chen et al., 1993). It is believed that cocaine-induced mu receptor upregulation is the result of changes in receptor trafficking rather than increases in gene transcription (Unterwald, 2001). The decrease in two proteins critical for mu opioid receptor internalization that was observed in the present study may reflect the mechanism of increased mu opioid receptors in response to chronic cocaine exposure. That is, constitutive mu receptor internalization may be decreased following chronic cocaine due to decreases in the levels of dynamin 2 and GRK2. Of note, both mu receptor upregulation and dynamin 2 and GRK2 down-regulation are dependent on D2 receptor activation. Similar to the mu opioid receptor upregulation that is seen following chronic cocaine exposure, opioid receptor antagonist treatment also results in increases in mu opioid receptors (Zukin et al., 1982; Yoburn et al., 1985; Unterwald et al., 1998). Mu receptor upregulation produced by opioid antagonists is also accompanied by decreases in dynamin 2 and GRK2 levels (Patel et al., 2002, 2003), further supporting the idea that decreases in the abundance of these trafficking proteins may decrease constitutive receptor cycling contributing to elevations in mu opioid receptor levels under both conditions.

The experiments described in this manuscript did not differentiate between the potential pre- or post-synaptic localization of cocaine-induced regulation of dynamin 2 and GRK2. The differential effects of D1 and D2 receptor antagonists on the changes in these trafficking proteins during acute versus chronic cocaine may be due to their cellular site of regulation. Both the D1 and D2 receptor antagonists blocked the dramatic upregulation of dynamin 2

and GRK2 seen in the accumbens following acute cocaine administration indicating that the regulation is occurring post-synaptically with or without a pre-synaptic component. As only the D2 receptor antagonist blocked the down-regulation seen after chronic cocaine, it is possible that the down-regulation of dynamin 2 and GRK2 is occurring pre-synaptically. Further experiments are necessary to establish the subcellular localization of this protein regulation.

Another finding of the present study is the brain region-selective nature of the regulation of dynamin 2 and GRK2 following both acute and repeated cocaine administration. Acute cocaine exposure had the most profound effect on dynamin 2 and GRK2 in the nucleus accumbens as compared to the other brain regions examined. The evidence that this effect is mediated by dopamine receptors suggests that plasticity of the dopamine synapse is an important response to cocaine and the resulting changes in synaptic dopamine concentrations. This plasticity may contribute to the critical role of the nucleus accumbens in cocaine-induced behaviors. In a similar manner, significant decreases in the levels of dynamin 2 and GRK2 were found following chronic cocaine administration only in the nucleus accumbens. Other studies have reported the brain-region specific regulation of DAT (Cass et al., 1993; Letchworth et al., 1997; Samuvel et al., 2008) and dopamine receptors (Goeders and Kuhar, 1987; Kleven et al., 1990; Unterwald et al., 1994) following chronic cocaine administration. For example, DAT protein binding is unaltered in the substantia nigra, but increased in the nucleus accumbens following chronic cocaine (Letchworth et al., 1997). Likewise, chronic binge-pattern cocaine results in an upregulation of D1 receptor in the accumbens but not the caudate putamen (Unterwald et al., 1994). These patterns of regulation of DAT and D1 receptors are consistent with the pattern of regulation shown herein; that is, DAT and D1 receptors are upregulated while dynamin 2 and GRK2 are down-regulated in the accumbens following chronic cocaine.

In conclusion, the data presented herein demonstrate that acute cocaine exposure resulted in a profound dopamine receptor-mediated increase in the expression of two proteins that play a role in modulating the synaptic landscape. Following chronic cocaine exposure, the effect is reversed and is mediated by dopamine D2 receptors. The regulation of dynamin 2 and GRK2 following both acute and chronic cocaine were most pronounced in the nucleus accumbens. These results suggest that receptor sequestration machinery plays a key role in the response to cocaine in a brain region critically important for mediating cocaine-induced behaviors. The differential effects of acute versus chronic cocaine on dynamin 2 and GRK2 expression in the nucleus accumbens indicates that protein trafficking may be a key factor underlying the initial and long-term adaptations that occur in response to cocaine exposure.

Acknowledgments

This work was supported by grants from the NIH: DA09580 (EMU), T32 DA07237 (EMU), and P30 DA13429 (MW Adler). The authors would like to thank Alwin Forbes for his technical contributions and Imran Sheikh and Jonathan Miller for their help with manuscript preparation.

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Figure 1.

Changes in dynamin 2 and GRK2 protein levels in response to 1, 3 or 14 days of binge-pattern cocaine administration are shown in the nucleus accumbens (A), caudate putamen (B), frontal cortex (C) and substantia nigra (D). The ratio of dynamin 2 or GRK2 to tubulin are shown as a percent of the levels in saline-injected control animals. Dynamin 2 and GRK2 levels were significantly elevated in the nucleus accumbens following 1 day of cocaine administration and were significantly decreased after 14 days of cocaine (A). GRK2 was significantly elevated following acute cocaine in the caudate putamen (B), while dynamin 2 was significantly reduced in the frontal cortex (C). No changes in either protein were observed after 3 days of cocaine in any brain region and at no time were these proteins altered in the substantia nigra (D). Data are expressed as mean + SEM; N= 6-8/treatment group; *** $p < 0.0001$, ** $p < 0.01$, * $p < 0.05$ as compared to controls injected with saline for the same number of days.

Figure 2.

Representative protein bands from Western blots showing changes in GRK2- (80 kDa, top) and dynamin 2- (100 kDa, bottom) immunoreactivity in rat nucleus accumbens following 1, 3 or 14 days of binge-pattern cocaine administration. S=saline; C=cocaine.

Figure 3.

Changes in dynamin 2 and GRK2 protein levels in the nucleus accumbens in response to 1 day of binge-pattern cocaine following pretreatment with either saline, SCH23390 (0.5 mg/kg ip), or eticlopride (1.0 mg/kg ip). The upregulation of dynamin 2 and GRK2 produced by acute cocaine (Saline + Coc) was significantly attenuated by pretreatment with either SCH23390 (SCH23390 + Coc) or eticlopride (Eticlopride + Coc). Dynamin 2 and GRK 2 were not significantly altered by either SCH23390 or eticlopride alone. Data are expressed as mean + SEM; N= 5-8/treatment group; ### p < 0.0001 as compared to Saline + Saline controls; ** p < 0.01 and *** p < 0.001 as compared to Saline + Cocaine.

Figure 4.

Changes in dynamin 2 and GRK2 protein levels in the nucleus accumbens following 14 days of binge-pattern cocaine. Daily cocaine administration was preceded by pretreatment with either saline, SCH23390 (0.5 mg/kg ip), or eticlopride (1.0 mg/kg ip). The down-regulation of dynamin 2 and GRK2 produced by chronic cocaine (Saline + Coc) was significantly attenuated by pretreatment with eticlopride (Eticlopride + Coc), but not SCH23390 (0.5 mg/kg). Dynamin 2 and GRK 2 were not significantly altered by either SCH23390 (SCH23390 + Saline) or eticlopride (Eticlopride + Saline) alone. Data are expressed as mean + SEM; N= 5-8/treatment group; # $p < 0.05$ as compared to Saline + Saline controls; * $p < 0.05$ and ** $p < 0.01$ as compared to Saline + Cocaine.

Figure 5.

Activity counts of animals injected with cocaine (15 mg/kg ip) in a binge-pattern on day 1 (left) and day 13 (right) of the study. Animals were injected with either saline, eticlopride (1.0 mg/kg ip), or SCH 23390 (0.5 mg/kg ip) at the -30 minute time point and cocaine at 0, 60, and 120 minutes. Pretreatment with either eticlopride or SCH23390 significantly attenuated the hyperactivity produced by binge-pattern cocaine administration (Day 1: $F[2,51] = 145.1$, $p < 0.0001$; posthoc tests $p < 0.001$. Day 13: $F[2,51] = 150.1$, $p < 0.0001$; posthoc tests $p < 0.001$). Activity is reported as the mean $\pm$ SEM of total number of beam breaks recorded during each ten minute period. $N=6-8$ /treatment group.