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Integrin expression is altered after acute and chronic cocaine

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Abstract

Cocaine addiction is associated with an increase in actin cycling and alterations in dendritic spines in the nucleus accumbens. Both actin polymerization and spine morphology are regulated in part by beta- (β) integrins. Mice were administered acute or daily injections of cocaine or saline for 7 days. After 3 weeks of withdrawal, the level of β -integrins in the postsynaptic density enriched subfraction from nucleus accumbens tissue was quantified by immunoblotting at 0, 30 or 120 minutes following an a cocaine challenge injection. After chronic treatment and withdrawal the basal level of $\beta 1$ -integrin was increased while $\beta 3$ -integrin was unaltered. However, following a cocaine challenge in chronic cocaine, but not saline-treated animals, $\beta 3$ -integrin was transiently up-regulated while $\beta 1$ -integrin was transiently downregulated. These data demonstrate a bidirectional regulation of β -integrins by chronic cocaine treatment that may contribute to cocaine-induced changes in actin cycling and dendrite morphology.

Keywords

cocaine; accumbens; integrin; sensitization

Integrins are heterodimer cell adhesion molecules consisting of an alpha (α) and beta (β) subunit that support the interface between cell membranes and the extracellular matrix [20]. Integrins can be found in synapses and can bidirectionally transduce signals between the extracellular and intracellular environments [4]. Several subunits are localized to the postsynaptic density (PSD) of neurons, including $\beta 1$, $\beta 3$, $\beta 5$ and $\alpha 5$ integrin subunits [4,6,16,18]. In the brain, $\beta 1$ -integrin is the most abundant and best studied subunit and has been shown to mediate outside in signaling through focal adhesion kinase[2,21]. Beta1-integrins promote actin polymerization in the hippocampus following theta burst induced long-term potentiation, and inhibiting $\beta 1$ -integrin signaling inhibits actin reorganization, as well as the induction of long term potentiation in response to theta burst stimulation [12–14]. Actin cycling in the nucleus accumbens is augmented after withdrawal from chronic cocaine administration and has been identified as a compensatory neuroadaptation regulating cocaine seeking behavior [19]. Actin cycling refers to the dynamic polymerization and disassembly between actin filaments, F-actin and monomeric or globular actin (G-actin) [15,17]. Given the capacity of integrin receptor stimulation to regulate actin cycling we hypothesized that β -integrin expression in the nucleus accumbens would be altered by acute and chronic cocaine administration.

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Male mice (C57, 8–10 week old) were purchased from *Harlan Laboratories* and housed 4 animals to a cage with food and water provided ad libitum using a 12 hr light/dark cycle with the light cycle starting at 6 am. All experiments were conducted in accordance with the *National Institutes of Health Guidelines for the Care and Use of Laboratory Animals*. On days 1 and 7 mice were injected with 15 mg/kg cocaine and on days 2–6 30mg/kg was administered. The control group received daily injections of saline. All animals remained in their home cages for 21–28 days of withdrawal. Fifteen mg/kg of cocaine was administered on days 1, 7 and 35 because this dose produces submaximal locomotor activity, thereby allowing locomotor sensitization to be more readily quantified. In a group of mice separate from those used for protein measurements, this treatment protocol resulted in greater locomotor activity on day 7 and 35 than on the first day of cocaine administration during the first 10 min after acute cocaine administration (Figure 1A). For protein measurements in the nucleus accumbens on days 21–28 of withdrawal, mice were decapitated immediately ($t = 0$), or at 30 or 120 min after a cocaine challenge injection (30 mg/kg, ip). For $t = 0$, animals were stuck with a syringe needle immediately prior to decapitation in order to provide a parallel handling procedure with those given cocaine and decapitated 30 or 120 min later. The nucleus accumbens was dissected and homogenized from each mouse and subfractionated as previously described to yield a postsynaptic density enriched fraction [19]. All buffers contained complete mini-tablets and phosphatase inhibitor cocktail (Pierce, Rockford, IL) to prevent protein degradation and dephosphorylation. Homogenized tissue was centrifuged twice at 1000g to remove the nuclear fraction and extracted once with a buffer containing 0.5 % triton X and centrifuged for 20 min at 12000g. The PSD enriched pellet was dissolved in a buffer containing 1% Triton X and 1% SDS [19]. Protein concentrations were assessed by BCA assay and the samples were run on 10% gels under normal Western blotting conditions. Antibodies utilized include $\beta 1$ -integrin (Chemicon, Billerica, MA), $\beta 3$ -integrin (Upstate, Billerica, MA), and phosphorylated focal adhesion kinase (Stressgen, Ann Arbor, MI), a signaling protein known to mediate integrin signaling and to regulate actin cycling [2, 21].

Chronic cocaine treatment followed by withdrawal resulted in a significant increase in $\beta 1$ -integrin expression in the PSD enriched fraction in comparison to chronic saline treatment (Figure 1B). There were no significant differences in $\beta 3$ -integrin or phosphorylated tyrosine kinase focal adhesion kinase between chronic cocaine and saline treatment groups.

Integrin expression in the accumbens was markedly altered by a cocaine challenge in chronic cocaine withdrawn mice. Beta1-integrin protein expression was significantly decreased 30 minutes after an acute cocaine injection and approached baseline by 120 minutes (Figure 2A). Correspondingly, a cocaine challenge returned beta 1 integrin protein levels to the level of expression observed in the chronic saline group without a challenge. Conversely, $\beta 3$ -integrin levels were increased 30 minutes following a challenge injection in the PSD enriched fraction (Figure 2B). Although, post hoc tests did not reveal a significant difference between time 0 and 30 minutes in $\beta 3$ -integrin levels, a significant difference was identified between time 30 and 120. Animals receiving chronic saline treatment did not show a significant effect of acute cocaine. However, there was a trend toward increase ($F_{(2,17)}=2.953$; $p= 0.07$) in $\beta 3$ -integrin expression over 120 minutes after an acute cocaine injection (Figure 2B).

These data indicate that changes in level of integrins in the nucleus accumbens may contribute to the neuroplasticity associated with withdrawal from chronic cocaine. The basal levels of $\beta 1$ -integrin were elevated after chronic cocaine, and $\beta 1$ - and $\beta 3$ -integrins were differentially regulated by a cocaine challenge injection only in animals withdrawn from chronic cocaine. The fact that this bidirectional change in integrin subunits occurred by 30 min and returned to basal levels by 120 min after injection approximates the behavioral effect of cocaine. Also, the fact that the changes in integrin levels were induced by cocaine only in chronic cocaine treated animals supports potential involvement of these changes in the behaviors that are produced by

chronic cocaine administration, such as locomotor sensitization. However, due to the difference in dosage between the behavioral and biochemical assays of sensitized animals it is uncertain if these changes are related.

Whether or not the changes in integrin levels contribute to the cocaine-induced behavioral changes requires further study. However, inhibition in $\beta 1$ -integrin reduces actin polymerization and restructuring [13], and the increased actin cycling induced by chronic cocaine is thought to be important in drug seeking since inhibiting actin cycling promotes cocaine-seeking in the reinstatement animal model [19]. This suggests that the chronic cocaine-induced upregulation of $\beta 1$ -integrin protein expression may promote actin cycling. However, the subsequent reduction in $\beta 1$ -integrin induced by a cocaine challenge would be predicted to facilitate cocaine-seeking by reducing actin cycling.

In addition to actin, integrins are known to regulate the surface expression of AMPA receptors. For example, reducing $\beta 1$ -integrin expression or function decreases electrophysiological measures of AMPA receptor expression and the capacity to induce LTP [5]. AMPA signaling and surface expression of GluR1 are augmented in the nucleus accumbens after withdrawal from chronic cocaine [3,11], and it is possible that the elevation in basal $\beta 1$ -integrin levels by chronic cocaine may contribute to this cocaine-induced change. The bidirectional expression of $\beta 1$ - and $\beta 3$ -integrins may be linked to the basal expression of AMPA receptors GluR1 and GluR2, respectively, because after cocaine self administration and withdrawal GluR1 surface expression is increased and GluR2 surface expression is unchanged [9]. Moreover, a cocaine challenge injection in chronic cocaine treated animals further elevates the surface expression of AMPA receptors at 30 minutes after injection [1]. Although this seems inconsistent with a $\beta 1$ -integrin regulation of AMPA receptor expression since $\beta 1$ -integrin levels were reduced at 30 min after a cocaine injection, $\beta 3$ -integrins also promote AMPA signaling by reducing internalization of AMPA receptors [8], posing a possible contribution by the elevation in $\beta 3$ -integrin levels at 30 minutes after injection to cocaine-induced augmented AMPA signaling. Finally, in noncontingent cocaine animals a cocaine challenge did not change phosphorylated GluR2 protein expression [10] which promotes the internalization of GluR2 containing AMPA receptors [7]. Taken together these studies pose the possibility that a change in $\beta 1$ -integrin expression may lead to upregulated GluR1 surface expression and an increase in $\beta 3$ -integrins may promote the stabilization of GluR2-containing receptors in the membrane. However, more experiments must be conducted to validate these hypotheses.

In conclusion, withdrawal from chronic cocaine produces changes in the basal level of $\beta 1$ -integrin in the nucleus accumbens, and results in bidirectional changes in $\beta 1$ - and $\beta 3$ -integrins during the first 30 min after a subsequent cocaine challenge injection. Previous studies with cocaine reveal marked changes by chronic cocaine in AMPA receptors and actin cycling, both of which have been linked to the behavioral changes induced by chronic cocaine [9,19]. While further experimentation is necessary to prove a causal relationship, the fact that integrins are known to regulate both AMPA receptor signaling and actin polymerization poses this class of cell adhesion molecules as potential mediators of chronic cocaine-induced plasticity and behavior.

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Figure 1. Behavior and protein expression after chronic treatment

(A) Sensitized locomotor behavior in response to repeated cocaine administration for 7 days and 3 weeks of withdrawal. Total distance was measured in 10-minute bins. A two-way ANOVA with repeated measures over time revealed significant effects of day ($F_{(2,204)} = 12.85$; $p = 0.003$) and time ($F_{(17,204)} = 51.36$; $p < 0.001$), and interaction between day and time ($F_{(34,204)} = 17.30$; $p < 0.001$). Arrow indicates time of cocaine injection (15 mg/kg, ip). (B) Integrin and phosphorylated focal adhesion kinase (p-FAK) protein expression after chronic saline or cocaine treatment and 3–4 weeks withdrawal ($t_{(16)} = 3.48$; $p = 0.003$). * $p < 0.05$, comparing days 7 and 35 to day 1 in each time bin in panel A using a Bonferroni post hoc test, or chronic saline to chronic cocaine in panel B.

Figure 2. Integrin expression was transiently altered after chronic cocaine treatment in the nucleus accumbens

(A) Beta1-integrin protein expression was reduced at 30 min after a cocaine challenge injection. A 2 way ANOVA was performed finding the interaction between time and treatment ($F_{(2,37)}=3.66$ $p=0.035$) and treatment effect ($F_{(1,37)}=8.02$ $p=0.007$) significant. (B) Beta3-integrin protein expression was increased at 30 min after a cocaine challenge injection. A 2 way ANOVA revealed a significant interaction ($F_{(2,36)}=3.79$ $p=0.032$), and effects of treatment ($F_{(1,36)}=11.13$ $p=0.002$) and time ($F_{(2,36)}=3.90$ $p=0.029$). * $p < 0.05$, compared to time= 0 or needlestick using a Bonferroni post hoc; + $p < 0.05$, compared to time= 120 min.