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κ-Opioid Receptor Activation Modifies Dopamine Uptake in the Nucleus Accumbens and Opposes the Effects of Cocaine

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Coadministration of κ-opioid receptor agonists (κ-agonists) with cocaine prevents alterations in dialysate dopamine (DA) concentration in the nucleus accumbens (Acb) that occur during abstinence from repeated cocaine treatment. Quantitative microdialysis was used to determine the mechanism producing these effects. Rats were injected with cocaine (20 mg/kg, i.p.), or saline, and the selective κ-agonist U-69593 (0.32 mg/kg, s.c.), or vehicle, once daily for 5 d. Extracellular DA concentration (DAext) and extraction fraction (Ee), an indirect measure of DA uptake, were determined 3 d later. Repeated cocaine treatment increased Ee, whereas repeated U-69593 treatment decreased Ee, relative to controls. Coadministration of both drugs yielded intermediate Ee values not different from controls. In vitro DA uptake assays confirmed that repeated U-69593 treatment produces a dose-related, region-specific decrease in DA uptake and showed that acute U-69593 administration increases DA uptake in a nontransporter reversible manner. Repeated U-69593 also led to a decrease in [125I]RTI-55 binding to the DA transporter (DAT), but did not decrease total DAT protein. These results demonstrate that κ-opioid receptor activation modulates DA uptake in the Acb in a manner opposite to that of cocaine: repeated U-69593 administration decreases the basal rate of DA uptake, and acute U-69593 administration transiently increases DA uptake. κ-agonist treatment also alters DAT function. The action of κ-agonists on DA uptake or DAT binding, or both, may be the mechanism(s) mediating the previously reported “cocaine-antagonist” effect of κ-opioid receptor agonists.

Key words: κ-opioid receptors; dopamine; dopamine uptake; cocaine; nucleus accumbens; striatum; quantitative microdialysis; rotating disk electrode voltammetry; autoradiography; Western blot; rats

Acute cocaine administration increases extracellular dopamine (DA) levels in the nucleus accumbens (Acb) by blocking the DA transporter and inhibiting DA clearance (Reith, 1988; Ritz et al., 1990). This action underlies the reinforcing effects of cocaine and may lead to the development of compulsive drug use (Wise and Bozarth, 1987; Kuhar et al., 1991). A marked enhancement of the psychomotor stimulant and rewarding effects of cocaine occurs after repeated cocaine administration (Lett, 1989; Stewart and Badiani, 1993; Shippenberg and Heidbreder, 1995; Schenk and Partridge, 1997). This phenomenon, referred to as behavioral sensitization, persists for weeks after cessation of cocaine use and is implicated in the reinstatement of cocaine-seeking behavior (Kalivas and Duffy, 1993a; Henry and White, 1995; Shippenberg and Heidbreder, 1995). Behavioral sensitization is associated with an increase in firing rate of mesolimbic DA neurons (Henry et al., 1989), an elevation of basal DA dialysate levels (Kalivas and Duffy, 1993a,b; Heidbreder et al., 1996), and an increase in the basal rate of DA uptake (Ng et al., 1991; Parsons et al., 1991; Meiergerd et al., 1994; Jones et al., 1995). These adaptations in presynaptic DA activity are thought to contribute to the development and long-term expression of behavioral sensitization (Kalivas and Stewart, 1991).

Coadministration of κ-opioid receptor agonists (κ-agonists) with cocaine prevents cocaine-induced behavioral sensitization (Heidbreder et al., 1993; Shippenberg et al., 1996) and the increase in basal DA dialysate levels that occur during cocaine abstinence (Heidbreder and Shippenberg, 1994). Acute administration of κ-agonists decreases DA levels in the Acb (DiChiara and Imperato, 1988; Donzanti et al., 1992; Maisonneuve et al., 1994), and some have hypothesized that this action of κ-agonists, which functionally opposes the acute effect of cocaine, underlies the cocaine-antagonistic effect of repeated κ-agonists.

Changes in dialysate concentration of a neurotransmitter are traditionally attributed to changes in extracellular concentration that are secondary to changes in release. However, recovery of monoamines by the dialysis probe has been shown to vary directly with the rate of monoamine clearance: increases in DA uptake increase DA recovery, and decreases in DA uptake reduce DA recovery (Bungay et al., 1990; Smith and Justice, 1994; Cosford et al., 1996). Therefore, changes in dialysate monoamine concentration may be caused by changes in either release or clearance, or both. The present study was conducted to test the hypothesis that κ-agonists prevent cocaine-induced changes in DA uptake. The net flux microdialysis method (Lönnroth et al., 1987) was used to characterize basal DA dynamics in rats after repeated administration of cocaine, the selective κ-agonist U-69593 (Lahti et al., 1985), or cocaine in combination with U-69593. This method provides an unbiased estimate of extracellular DA concentration and an indirect measure of DA uptake (Bungay et al., 1990; Justice, 1993). Rotating disk electrode voltammetry, autoradiography, and immuno blotting were then used, respectively, to directly assess the influence of U-69593, in the presence or absence of cocaine, on the rate of DA uptake, binding to the DA transporter (DAT), and total DAT protein in Acb and dorsal striatum (STR).
samples were collected between 8 A.M. and 1 P.M. The net flux method of microdialysis as described by Justice (1993). Samples were placed into the microdialysis chamber (40 x 40 x 35 cm) in a separate room where the temperature, light, and feeding conditions were controlled. A 4 mm section from the anterior portion of the brain was removed (beginning 3 mm posterior to the anterior pole) with the aid of a metal brain matrix. A 2 mm coronal section was placed on an ice-cold plate, and the Acb was removed bilaterally by dissecting a 3 mm section surrounding the anterior commissure, which typically yielded 10–15 mg of tissue. The STR was removed by cuttting out a 4 mm round disc from the center of the left or right STR. Only one STR was used (left and right sampled alternately).

Microdialysis Procedure. Rats (n = 14, 250–350 gm) were treated once per day for 5 d with U-69593 (0.32 mg/kg, s.c.) or vehicle. Three days after the last treatment, rats were decapitated, the brains were rapidly removed, and the Acb and STR were rapidly dissected to analyze the rate of DA uptake by HPLC and electrochemical detection. The DA uptake assay was conducted in follow-up: additional groups of rats were treated with either varying doses of U-69593 (0.03, 0.1, or 0.32 mg/kg, s.c.) or vehicle once per day for 5 d, and DA uptake was assessed by RDE voltammetry 3 d later (n = 20–30/group). One group was treated with vehicle, one group of U-69593 (0.32 mg/kg, s.c.) or vehicle once per day for 5 d, and changes in the kinetics of DA uptake were assessed using RDE voltammetry 3 d later (n = 22, 250–300 gm). In the latter experiment, procedures described in Povlock and Schenk (1997) were used to construct a Michaelis-Menten expression from which the V_{max} and K_m were determined.

Dissections. A 4 mm section from the anterior portion of the brain was removed (beginning 3 mm posterior to the anterior pole) with the aid of a metal brain matrix. A 2 mm coronal section was placed on an ice-cold plate, and the Acb was removed bilaterally by dissecting a 3 mm section surrounding the anterior commissure, which typically yielded 10–15 mg of tissue. The STR was removed by cuttting out a 4 mm round disc from the center of the left or right STR. Only one STR was used (left and right sampled alternately).

DA uptake assay. After dissection, the Acb and STR tissues were quickly weighed, placed into 1.5 ml microcentrifuge tubes and stored in 50 μl of 4°C physiological saline buffer until the start of assay (5 min and 25 min later for the Acb and STR, respectively). The buffer consisted of (in mM): 124 NaCl, 3 KCl, 1.24 KH2PO4, 1.30 MgSO4, 2.5 NaHCO3, 26.0 CaC2O4, and 10 d-glucose, pH 7.4, and gassed with 5% O2–95% CO2 in a 95°C bath. The tissue was washed twice in 2 ml of the ice-cold buffer and then replacing 400 μl of the ice-cold buffer. Next, 400 μl of the cold buffer was replaced with room temperature buffer, and the tissue solution was increased to 37°C over 5 min. The tissue was carefully removed from the microcentrifuge tube with a glass dish and placed in 300 μl of ice-cold buffer. The miniced tissue was placed into an electrochemical cell with 300 μl of 37°C buffer. During the assay, the tissue was maintained at 37°C, subjected to a constant current of 95% O2/5% CO2 gas directed over the top of the electrochemical cell, and stirred at 480 rpm.

Voltammetric measurements were made before (~5 min) and for 1 min after a 6 μl addition of DA (100 μM in buffer). The electrochemical cell and the supporting RDE voltammetry system were described by Burnette et al. (1996) and Welch and Justice (1996). The applied potential was +450 mV versus Ag/AgCl. Data acquisition and analysis were performed using Origin data acquisition software (MicroCal, Northampton, MA) on a 486 PC.

Data analysis. The velocity of DA uptake into Acb and STR tissue was calculated by linear regression of the data for each concentration using the rate of DA delivery from tissue and the rate of DA delivery to tissue. The initial rate of DA delivery from tissue was calculated using the concentration of DA uptake into the tissue from the initial velocity of DA uptake in each experiment and expressed as the initial velocity of DA uptake in picomoles per second per gram of wet tissue weight.

Kinetic parameters, V_{max} and K_m were determined by following the procedure described in Povlock and Schenk (1997). Acb tissue suspensions were exposed to increasing concentrations of DA (0.25, 0.5, 1, and 3 μM) for 5 min. After each concentration, the initial velocity was determined as described above. Each addition of DA took place when the current from the previous addition had returned to the original baseline level. This experimental design has been shown to produce conditions approximating the apparent low-to-infinite time experiment.
as characterized by Povlock and Schenk (1997). Values of \(K_a\) and \(V_{\text{max}}\) were estimated by fitting experimentally observed values of the initial velocity by each DA concentration to the Michaelis-Menten expression using commercially available nonlinear curve fitting software (Prism, San Diego, CA).

One-way ANOVA was used to compare the dependent measure (picomoles per second per gram of tissue weight or kinetic parameter) among treatments with appropriate specification of group differences indicated in post hoc analyses using a Newman–Keuls pairwise comparison of the group means test at \(p < 0.05\).

**Experiment 3: Influence of repeated U-69593 and cocaine treatment on [\(^{125}\)I]RTI-55 binding in Acb and STR**

**Procedure.** Rats (\(n = 16, 250–350\) gm) were treated once per day for 5 d with U-69593 (320 \(\mu\)g/kg, s.c.) or vehicle, and cocaine (20 mg/kg, i.p.) or vehicle. Three days after the last treatment, rats were killed by decapitation, and the brains were rapidly frozen in isopentane and stored at \(-80^\circ\text{C}\) until sectioned.

 Autoradiography. Frozen coronal sections (20 \(\mu\m) were cut, thaw-mounted onto chrome alum/gelatin-coated microscope slides, dried, and stored at \(-80^\circ\text{C}\) until processed for autoradiography. Two coronal sections within the caudoputamen region were mounted on each slide such that two slides (four consecutive sections) per animal were prepared for labeling with [\(^{125}\)I]RTI-55, the ligand used to selectively label the DATs. The brain sections were thawed to room temperature and preincubated for 30 min in 50 mM sodium phosphate buffer (NaH\(_2\)PO\(_4\), Na\(_2\)HPO\(_4\), pH 7.4) containing 0.1% bovine serum albumin and 1% BSA. Sections were then incubated at room temperature for 4 hr in the above buffer containing 1 \(\times\) protease inhibitor mixture (1 \(\times\) PIC) and 10 \(\times\) [\(^{125}\)I]RTI-55 (DuPont, NEN, specific activity 2200 Ci/mmol). Sections used to assess total binding were incubated in the same buffer medium with the addition of unlabeled citalopram (50 \(\mu\m) to block binding to the serotonin transporter. Nonspecific binding was assessed with the addition of unlabeled 100 \(\mu\m) imidazoline. After incubation the slides were washed for two 5 min periods in cold (4°C) sodium phosphate buffer (50 mM, containing 10 mM NaCl and 0.1% BSA), dipped in cold deionized water, and desiccated until completely dried (1 hr).

Labeled slides and microscale standards for both radioligands were exposed to Hyperfilm-\(^{3}H\) (both from Amersham, Arlington Heights, IL), and after 8 d of exposure at room temperature, the films were developed using commercially available x-ray developers and fixers.

**Data analysis.** A Macintosh Apple Power G3 computer and a scanner (Powerlook 3000 with UMAX software) were used to digitize the brain sections on film. The NIH Image 1.62 program (developed at the National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/) was used to construct the standard curves and to quantify relative optical densities of brain regions. The Rodbard curve, as characterized by Povlock and Schenk (1997). Values of \(K_a\) and \(V_{\text{max}}\) were estimated by fitting experimentally observed values of the initial velocity by each DA concentration to the Michaelis-Menten expression using commercially available nonlinear curve fitting software (Prism, San Diego, CA).

The thalamus and the globus pallidus were removed for analysis of DA uptake by RDE voltammetry. The Acb was removed for analysis of DA uptake by RDE voltammetry. The dose and treatment interval for nBNI was previously shown to result in the selective and long-lasting blockade of \(\kappa\)-opioid receptors (Horan et al., 1992; Spanagel et al., 1994).

**RESULTS**

**Effect of repeated U-69593 + cocaine treatment on \(D_{\text{ext}}\) and \(D_{\text{int}}\)**

Histological analysis confirmed placement of the microdialysis probe within the Acb in 93% of the subjects. In these rats (\(n = 47\)), the active length of the probe was observed to lie medially in the anterior-posterior extent of the Acb and to traverse the dorsalventral length of the Acb medial to the anterior commissure (Fig. 1). No systematic group differences in placement were observed.

The no net flux microdialysis method was used to study changes in presynaptic DA dynamics in vivo. In contrast to conventional methods, this method provides an estimate of extracellular analyte concentration and in vivo extraction fraction (Lönnroth et al., 1987; Lönnroth et al., 1989). Extraction fraction, or \(E_{\text{ext}}\), is a measure of the ability of the surrounding tissue to accept DA from the probe, and theoretical consideration of the dialysis process in neural tissue suggests an association between \(E_{\text{ext}}\) and changes in DA clearance (Bungay et al., 1990). This relationship has received support from empirical observations in which DA uptake, but not release or metabolism, modifies DA \(E_{\text{ext}}\) (Justice, 1993; Smith and Justice, 1994). Increasing DA uptake facilitates diffusion of DA from the probe into tissue and increases \(E_{\text{ext}}\), whereas decreasing DA uptake reduces diffusion of DA into the tissue and decreases \(E_{\text{ext}}\).

Figure 2 depicts the net no flux plot of the mean (±SEM) change in perfusate DA concentration (\(D_{\text{int}} - D_{\text{in}}\)) at each \(D_{\text{in}}\) and the average linear regression for each experimental group assessed in this experiment. Mean (+SEM) basal \(D_{\text{int}}\) and \(D_{\text{in}}\) are reported in Table 1. \(D_{\text{int}}\) varied from 9.9 ± 2.5 nm (U-69593 + cocaine) to 13.3 ± 2.1 nm (U-69593 + saline), but did not differ significantly among treatment groups (interaction, \(F_{(1,43)} < 1\)), U-69593 treatment main effect, \(F_{(1,43)} < 1\); cocaine treatment main effect, \(F_{(1,43)} < 1\). In contrast, main effects of cocaine treatment (\(F_{(1,43)} = 4.8, p = 0.03\)) and U-69593 treatment (\(F_{(1,43)} = 10.1, p = 0.003\)) were significant for \(E_{\text{ext}}\). After cocaine treatment, \(E_{\text{ext}}\) was significantly increased relative to control. In contrast, after U-69593 treatment, \(E_{\text{ext}}\) was significantly decreased relative to control. The interaction values for comparison in each brain region. Group differences were assessed statistically by one-way ANOVA followed by a Newman–Keuls pairwise comparison of the group means test at \(p < 0.05\).

**Experiments 5 and 6: Influence of acute U-69593 on DA uptake in Acb tissue**

**Procedure.** The rate of DA uptake in the Acb after a single injection of U-69593 was assessed in vitro by RDE voltammetry using the same procedures described in Experiment 2. In Experiment 4 (time course), rats (250–350 gm, \(n = 26\)) were injected with U-69593 (0.32 mg/kg, s.c.) or its vehicle, and the rate of DA uptake was determined 1, 2, or 4 hr later. In Experiment 5 (dose–response), rats (250–350 gm, \(n = 31\)) were treated with U-69593 (0.03, 0.10, or 0.32 mg/kg, s.c.) or its vehicle, and the rate of DA uptake was determined 2 hr later.

**Data analysis.** The initial rate of DA uptake was calculated as described in Experiment 2, and group differences by time or dose were assessed statistically by one-way ANOVA followed by a Newman–Keuls pairwise comparison of the group means test at \(p < 0.05\).

**Experiment 7: Influence of \(\kappa\)-opioid receptor blockade on the acute U-69593-induced increase in DA uptake in the Acb**

**Procedure.** Rats (\(n = 23, 350–450\) gm) were treated with a single injection of nor-binaltorphimine (nBNI) (10 mg/kg, s.c.) or vehicle (1 ml/ml sterile water), 24–48 hr before treatment with U-69593 (0.32 mg/kg, s.c.) or its vehicle. Rats were decapitated 2 hr after the U-69593 treatment, and the Acb was removed for analysis of DA uptake by RDE voltammetry. The dose and treatment interval for nBNI was previously shown to result in the selective and long-lasting blockade of \(\kappa\)-opioid receptors (Horan et al., 1992; Spanagel et al., 1994).

**Data analysis.** The initial rate of DA uptake was calculated as described in Experiment 2 and group differences were assessed statistically by two-way ANOVA (\(\kappa\)-agonist (nBNI or vehicle) × \(\kappa\)-antagonist (U-69593 or vehicle)) followed by simple effect probes.
between U-69593 and cocaine was not significant ($F_{(1,43)} < 1$). $E_d$ values in rats that had received U-69593 with cocaine (U-69593 + cocaine, 0.18 ± 0.03) were not different from controls (vehicle + saline, 0.22 ± 0.03).

Effect of repeated U-69593 treatment on DA uptake in Acb and STR

RDE voltammetry was used to directly measure the effect of U-69593 treatment on the rate of DA uptake in Acb. As predicted from the microdialysis experiment, the rate of DA uptake in the Acb was significantly reduced, relative to controls, in rats that had received the 5 d U-69593 treatment regimen (Fig. 3a) ($F_{(1,10)} = 6.8; p = 0.03$). The effect was dose-related (Fig. 3c) ($F_{(0.23)} = 3.2; p = 0.04$) and region specific, because no decrease in the rate of DA uptake was observed in STR tissue from these same rats (Fig. 3b) ($F_{(1,12)} = 3.1; p = 0.11$) regardless of dose (Fig. 3d) ($F_{(2,24)} < 1$).

The observed changes in the initial velocity of DA clearance after the U-69593 treatment were associated with significant increases in both $K_m$ ($F_{(1,20)} = 4.84, p < 0.04$) and $V_{max}$ ($F_{(1,20)} = 4.83, p < 0.04$) in the Acb (Table 2).

### Table 1. Basal extracellular DA concentration ($DA_{ext}$) and in vivo extraction fraction ($E_d$) in rat Acb 3 d after a 5 d treatment with cocaine with or without the selective $\kappa$-opioid receptor agonist U-69593

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$DA_{ext}$ (nM)</th>
<th>$E_d$</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle + saline</td>
<td>10.9 ± 2.1</td>
<td>0.22 ± 0.03</td>
<td>12</td>
</tr>
<tr>
<td>U-69593 + saline</td>
<td>13.3 ± 2.1</td>
<td>0.11 ± 0.01</td>
<td>10</td>
</tr>
<tr>
<td>Vehicle + cocaine</td>
<td>11.4 ± 1.7</td>
<td>0.31 ± 0.06</td>
<td>12</td>
</tr>
<tr>
<td>U-69593 + cocaine</td>
<td>9.9 ± 2.5</td>
<td>0.18 ± 0.03</td>
<td>13</td>
</tr>
</tbody>
</table>

$^a$U-69593 significantly decreased $E_d$ ($p < 0.05$ by main effect identified by two-way ANOVA).

$^b$Cocaine significantly increased $E_d$ ($p < 0.05$ by main effect identified by two-way ANOVA).

Effect of repeated U-69593 + cocaine on $[^{125}]$RTI-55 autoradiography in Acb and STR

$[^{125}]$RTI-55 binding (femtomoles per milligram of tissue), in the presence of unlabeled 50 nM citalopram to prevent binding to the serotonin transporter, was used to determine the influence of repeated U-69593 treatment alone or in combination with cocaine, on DAT binding density in the Acbshell, Acbcore, and dorsal STR. The mean (±SEM) binding density is given in Table 3 and illustrated in Figure 4. No significant interactions between U-69593 treatment and cocaine treatment were found ($F_{(1,12)} < 1$ in each analysis). However, in the Acb, a significant main effect of U-69593 on $[^{125}]$RTI-55 binding was found in both the shell and core regions ($F_{(1,12)} = 5.30, p = 0.04$ and $F_{(1,12)} = 5.01, p = 0.04$, respectively). In each region, repeated U-69593 treatment significantly decreased $[^{125}]$RTI-55 binding (33 and 35% in the shell and core, respectively). The decrease in $[^{125}]$RTI-55 binding was evident in both U-69593 + saline and U-69593 + cocaine tissues (Table 3). A similar trend, although not statistically significant, was observed in the dorsal STR ($F_{(1,12)} = 4.46, p = 0.056$). No differences as a result of cocaine administration were observed in any statistical analyses.
produced intermediate levels of DAT immunoreactivity not statistically different from either controls or U-69593 + cocaine treatment. Similar effects were not found in the STR (\(F_{(3,27)} = 1.6, p = 0.23\)).

**Effect of an acute U-69593 treatment on DA uptake in Acb**

In view of the changes in DA uptake after repeated treatment with U-69593, experiments were conducted to characterize the effect of acute U-69593 administration on DA uptake. Figure 6a shows the rate of DA uptake in Acb at various time points after a single U-69593 (0.32 mg/kg, s.c.) or vehicle injection. A significant time-dependent increase in the rate of DA uptake in the Acb was observed after U-69593 administration (\(F_{(6,42)} = 5.3, p = 0.007\)). The greatest increase in uptake was apparent 2 hr after the injection. Figure 6b shows that this effect was dose-related (\(F_{(3,27)} = 4.2, p = 0.02\)).

**Effect of nBNI on the acute action of U-69593 on DA uptake**

nBNI (10 mg/kg, s.c.), a \(\kappa\)-opioid receptor antagonist, was used to determine the role of \(\kappa\)-opioid receptors in mediating the acute effect of U-69593 on DA uptake in the Acb. A significant two-way interaction between nBNI treatment and U-69593 treatment was found (\(F_{(2,15)} = 6.67, p < 0.02\) (Fig. 6c)). nBNI treatment blocked the increased rate of DA uptake induced by a single U-69593 injection. No significant effect of nBNI alone on DA uptake was observed.
in contrast to repeated cocaine administration, repeated κ-agonist administration leads to a decrease in DA uptake and release. In rats that had received U-69593 with cocaine, \( E_d \) was intermediate to that produced by each drug alone and approximated control levels. Statistical analysis revealed significant main effects for U-69593 and cocaine treatments and no significant interaction, suggesting that each drug alone modified DA uptake. Thus, repeated activation of κ-opioid receptors during cocaine administration may prevent cocaine-induced alterations in DA neurotransmission by producing long-term alterations in basal DA uptake and release that are opposite to those produced by cocaine.

**DA uptake is decreased after repeated systemic U-69593 treatment**

Direct evidence to support the conclusion that repeated U-69593 treatment decreases DA uptake was obtained using RDE voltammetry that measures DA uptake *in vitro*. The initial rate of DA uptake in the Acb from U-69593-treated rats was significantly lower than in vehicle-treated controls. The magnitude of the effect was dose dependent, and the effective doses (0.1 and 0.32 mg/kg, i.p.) were those previously shown to block behavioral sensitization to cocaine (Heidbreder et al., 1993; Shippenberg et al., 1996). DA uptake was unaltered in STR tissue, suggesting that the effect of U-69593 administration on DA uptake is region specific (Fig. 3). The changes in uptake observed in the Acb were associated with a significant increase in \( K_m \) (37%) and \( V_{\text{max}} \) (15%) (Table 2). The change in \( K_m \) is consistent with the observed decrease in DA uptake; however, this effect is counteracted at least in part by an increase in \( V_{\text{max}} \). Further analysis of the kinetic consequences of repeated κ-agonist treatment would require additional information such as the number of functional transporters.

**DAT binding and total DAT protein are altered after repeated systemic U-69593 treatment alone, or in combination with cocaine**

Repeated U-69593 treatment also reduced \([^{125}\text{I}]\text{RTI-55}\) binding in the core and shell of the Acb (Fig. 4). These results provide the first demonstration that repeated U-69593 treatment downregulates DAT in the Acb and suggest that U-69593-induced decreases in DA uptake may result from a decrease in DAT activity. Preliminary studies suggest that the U-69593 treatment regimen decreases the \( B_{\text{max}} \) of \([^{3}H]\text{WIN 35,428} \) (Izenwasser et al., 1997) or \([^{125}\text{I}]\text{RTI-55}\) (Sharpe et al., 1999) to DAT in the Acb. These effects, however, do not appear to be mediated by loss of protein, because immunoblotting revealed no decrease in DAT immunoreactivity after U-69593 treatment. Alternatively, repeated U-69593 treatment may lead to post-translational modifications of DAT that affect
antagonist binding and DA uptake. For example, repeated $\kappa$-agonist treatment is associated with an upregulation of protein kinase C (PKC) activity in brain tissue (Feng et al., 1996). Activation of PKC is known to increase DAT phosphorylation (Huff et al., 1997; Vaughan et al., 1997), promote its internalization, and decrease DA uptake (Daniels and Amara, 1999; Melikian and Buckley, 1999), consistent with the results observed on DA uptake here. Internalization of DAT may also affect its availability to bind $^{125}$I-JRTI-55 and DA, leading to decreased DAT binding in the absence of a decrease in total DAT protein. Protein kinase C is also activated by $\kappa$-opioid receptor stimulation, as are other signaling cascades (Belcheva et al., 1998; Zhang and Wong, 1998; Bohn et al., 2000). However, whether these effects mediated the observed changes in DA uptake after acute $\kappa$-agonist treatment is unclear.

Repeated administration of cocaine increased DA uptake but produced no changes in total DAT protein or $^{125}$I-JRTI-55 binding to DAT. This suggests that the increases in DA uptake observed during the early phase of cocaine abstinence (Parsons et al., 1991; Meiergerd et al., 1994) (see Experiment 1 here) are not caused by an upregulation in DAT. In contrast, repeated administration of cocaine in combination with U-69593 produced a significant decrease in DAT binding to $^{125}$I-JRTI-55 and a significant increase in total DAT protein in the Acb (Figs. 4, 5). It is unclear how increases in DAT expression, in the face of decreased transporter binding, can account for the normalization of DA uptake observed in rats receiving repeated cocaine with U-69593. However, increasing evidence suggests that changes in DAT function (e.g., increases or decreases in DA uptake) can be dissociated from changes in protein expression and ligand recognition sites (Kitayama et al., 1992; Lee et al., 1996; Kokoshka et al., 1998).

**REFERENCES**


