Lack of Cocaine Effect on Dopamine Clearance in the Core and Shell of the Nucleus Accumbens of Dopamine Transporter Knock-Out Mice

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Cocaine produces its reinforcing effects primarily by inhibiting the dopamine transporter (DAT) at the level of presynaptic terminals and increasing extracellular levels of dopamine (DA). Surprisingly, in mice genetically lacking the DAT, cocaine was still able to elevate DA in the nucleus accumbens (NAc). This finding is critically important for explaining the persistence of cocaine reinforcement in DAT knock-out (DAT-KO) mice. However, the mechanism by which cocaine elevates DA is unclear. Here, we tested the recently proposed hypothesis that in the absence of the DAT, the norepinephrine transporter (NET) could provide an alternative uptake site for DA clearance. If true, cocaine could elevate DA levels through its inhibition of the NET. *In vitro* voltammetry, a technique well suited for evaluating the effects of drugs on DA uptake, was used in the present study. We report that both cocaine and desipramine, a potent NET inhibitor, failed to change DA clearance or evoked release in the NAc of mutant mice. Additionally, fluoxetine, a serotonin transporter (SERT) inhibitor, also had no effect on these parameters. These data rule out the involvement of accumbal NET or SERT in the cocaine-induced increase in extracellular DA in DAT-KO mice. Moreover, the present findings suggest that in the DAT-KO mice, cocaine acts primarily outside the NAc to produce its effects.

Key words: cocaine; nucleus accumbens; dopamine; DAT knock-out mice; desipramine; voltammetry

Addictive drugs have the common property of elevating dopamine (DA) levels in the striatum, and this effect is more pronounced in the nucleus accumbens (NAc) (Carboni et al., 1989; Cass et al., 1992; Wu et al., 2001). Cocaine elevates DA in this region by blocking the uptake of DA through the DA transporter (DAT) (Ritz et al., 1987). It is commonly believed that the ability of cocaine to inhibit the DAT is directly related to its reinforcing properties (Ritz et al., 1987; Koob and Bloom, 1988; Kuhar et al., 1991; Volkow et al., 1997). A high degree of correlation was found between the potency of cocaine-like drugs as inhibitors of DA uptake and their propensity to be self-administered (Ritz et al., 1987; Madras et al., 1989). Surprisingly, in knock-out mice with a genetic deletion of the DAT (DAT-KO mice), cocaine-conditioned place preference (Sora et al., 1998, 2001) and self-administration of cocaine (Rocha et al., 1998) were still observed. This could argue against a primary role of DA in cocaine reinforcement. However, recent microdialysis studies have found that in the absence of the DAT, cocaine may still increase the levels of extracellular DA in the NAc (Carboni et al., 2001), although not in the dorsal striatum (Rocha et al., 1998; Carboni et al., 2001). This finding is critical not only for explaining cocaine reinforcement in DAT-KO mice but also for support of the DA hypothesis of reward. The mechanism postulated to elevate DA is a decrease in the clearance rate of DA by cocaine via norepinephrine transporter (NET) inhibition. Although in normal mice, NET does not take up DA in the NAc, NET uptake of DA may be a compensatory mechanism that takes place in the NAc of DAT-KO mice (Carboni et al., 2001). This hypothesis was supported by the finding that reboxetine, a NET inhibitor, increased dialysate DA levels in the NAc of DAT-KO mice but not of wild-type mice (Carboni et al., 2001). However, because extracellular DA is regulated by multiple factors, including release, uptake, and metabolism, a direct assessment of the effect of cocaine on DA clearance is necessary to test this possibility.

The present study was designed to test whether cocaine slows the clearance of DA in the NAc of DAT-KO mice. *In vitro* fast-scan cyclic voltammetry (FSCV) allowed examination of the effect of cocaine on DA dynamics in both the core and shell of the NAc in DAT-KO mice.

MATERIALS AND METHODS

Animals. Homozygote DAT-KO and wild-type littermate mice derived from the crossing (more than 30 generations) of heterozygous DAT 129SvJ/C57BL mice, as described previously (Giros et al., 1996), were

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used for this study. Animals were housed three to five per cage on a 12 hr light/dark cycle with *ad libitum* access to water and food. All animal procedures were approved by the institutional animal care and use committee.

Cyclic voltammetry in brain slices. Mice were decapitated, and the brains were rapidly removed and cooled in ice-cold, pre-oxygenated (95% O_2/5% CO_2), modified Krebs' buffer. The tissue was then sectioned with a vibrating tissue slicer (Leica VT1000S; Leica Instruments, Nussloch, Germany) into 400-μm-thick coronal slices containing the NAc. Slices were kept in a reservoir of oxygenated Krebs' buffer at room temperature until required. Thirty minutes before each experiment, a brain slice was transferred to a "Scottish-type" submersion recording chamber, perfused at 1 ml/min with 34°C oxygenated Krebs', and allowed to equilibrate. The Krebs' buffer consisted of (in mM): NaCl 126, KCl 2.5, NaH_2PO_4 1.2, $CaCl_2$ 2.4, $MgCl_2$ 1.2, $NaHCO_3$ 25, glucose 11, HEPES 20, and L-ascorbic acid 0.4; pH was adjusted to 7.4. DA was evoked by a single, rectangular, electrical pulse (300 μA, 2 msec per phase, biphasic), applied every 10 min. DA was detected using FSCV as described earlier (Jones et al., 1996, 1998; Budygin et al., 2001). Once the extracellular DA response to electrical stimulation was stable for three successive stimulations, cocaine, fluoxetine, or desipramine (Sigma-RBI, St. Louis, MO) was applied to the NAc via the superfusate. A concentration of 10 μM cocaine was chosen to mimic the maximal peak brain concentration after a dose of 20 mg/kg given intraperitoneally (Nicolaysen and Justice, 1988). Fluoxetine and desipramine were applied at the same concentration (10 μM). Each test was performed in one slice, which served as its own precondition control. For each experimental group, slices were obtained from at least five animals.

Data analysis. Background subtracted cyclic voltammograms were constructed by subtracting the background current obtained before release from the current measured after release. In each case, DA was the substance detected, and it was identified by its characteristic cyclic voltammogram. The oxidation current for DA was converted to concentration by electrode calibration with 10 μM DA at the end of the experiment. Measured time courses of DA were analyzed with a Michaelis–Menten-based set of kinetic equations (Wightman et al., 1988) to determine the concentration of DA detected and the rate of DA transport. Time courses in DAT-KO mice were evaluated as a pseudo first-order rate constant (k). To compare kinetics between genotypes, a rate constant k was calculated by dividing V_{max} by K_m values in wild-type mice (Jones et al., 1998).

Statistics. Statistical analyses using paired and unpaired Student's t tests were performed with GraphPad Prism (GraphPad Software, San Diego, CA). The data are presented as mean ± SEM. Differences with $p < 0.05$ are reported.

RESULTS

DA was monitored by fast-scan cyclic voltammetry, and release and uptake parameters were calculated from these traces. The rate of DA clearance, reported as a rate constant k (calculated as a first-order rate constant using the formula V_{max}/K_m), was 200 times slower in NAc core (0.04 sec^{-1} vs 8.0 sec^{-1}; $p < 0.0001$; $n = 6$) and 130 times slower (0.03 sec^{-1} vs 4.0 sec^{-1}; $p < 0.0001$; $n = 8$) in NAc shell of DAT-KO mice as compared with wild-type mice (Fig. 1). Although there is no difference in clearance rate of DA between the NAc core and shell in slices from DAT-KO mice ($p > 0.05$), the clearance rate constant in the NAc shell of slices from wild-type mice is approximately half that of the NAc core (4.0 sec^{-1} vs 7.8 sec^{-1}; $p < 0.05$) (Fig. 1). Application of 10 μM cocaine for 20 min prolonged the clearance of dopamine in both the core (7.8 sec^{-1} vs 0.3 sec^{-1}; $p < 0.005$; $n = 6$) and shell (4.0 sec^{-1} vs 0.2 sec^{-1}; $p < 0.005$; $n = 7$) NAc in slices from wild-type animals (Figs. 1, 2). There were no significant changes in single pulse-evoked DA release after the drug in either the shell (0.54 ± 0.09 vs 0.48 ± 0.11 μM; $p > 0.05$; $n = 7$) or core (0.89 ± 0.24 vs 1.00 ± 0.29 μM; $p > 0.05$; $n = 6$) NAc of wild-type mice. The clearance rate constant (Figs. 1, 2) and evoked DA release (0.41 ± 0.24 vs 0.39 ± 0.17 μM, $n = 6$ for core; 0.36 ± 0.06 vs 0.40 ± 0.09 μM, $n = 9$ for shell) in NAc slices from DAT-KO animals were unaltered by cocaine ($p > 0.05$). Desipramine (10 μM) had no effect on either DA clearance (Fig. 3*A*) or DA release (0.36 ± 0.06 vs 0.28 ± 0.05 μM; $p > 0.05$; $n = 6$) in the shell of the NAc from DAT-KO or wild-type animals (data not shown). Similarly, 10 μM fluoxetine did not alter either DA clearance (Fig. 3*B*) or DA release (0.45 ± 0.10 vs 0.44 ± 0.14 μM) in DAT-KO mice ($p > 0.05$; $n = 6$).

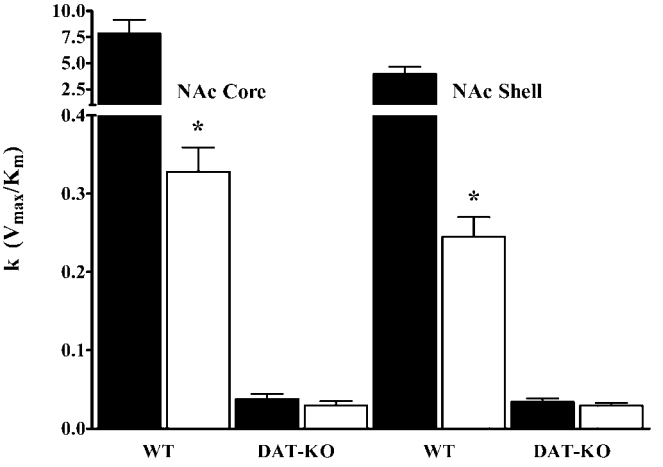

Figure 1. Effect of cocaine on DA clearance in the core and shell NAc in wild-type (*WT*) and DAT-KO mice. The rate of DA clearance, reported as a rate constant k, is significantly decreased by cocaine in both the core and shell of the NAc in wild-type mice ($^*p < 0.005$). Cocaine had no effect on DA clearance in either the NAc core or shell of DAT-KO mice ($p > 0.05$). *Filled bars*, Control; *open bars*, 20 min application of 10 μM cocaine.

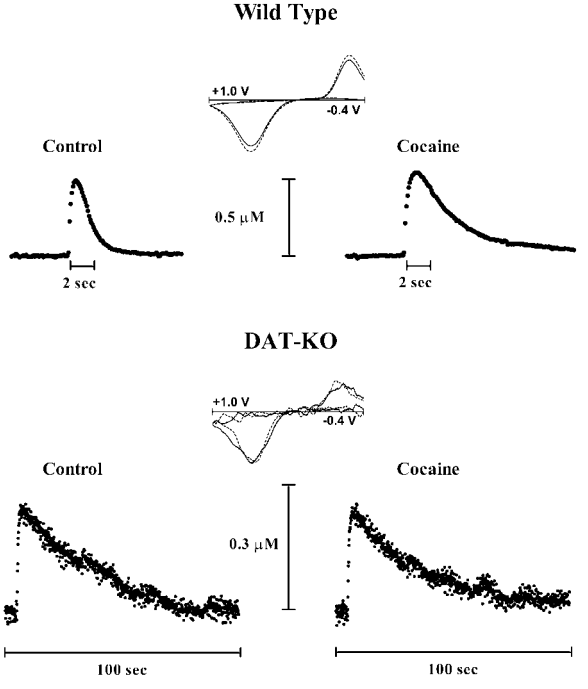

Figure 2. Cocaine slows DA clearance in NAc shell of wild-type but not DAT-KO mice. The effect of cocaine on evoked DA efflux in the shell of NAc in wild-type (*top*) and DAT-KO (*bottom*) mice is shown. Locally evoked (single 300 μA, 2 msec per phase, biphasic pulse) DA overflow was measured by FSCV in NAc shell slices before (*left*) and during (*right*) cocaine (10 μM) bath application (20 min). *Insets* are background-subtracted cyclic voltammograms taken at the peak response. There is an oxidation peak at 600 mV and a reduction peak at −200 mV versus Ag/AgCl, identifying the released species as DA. *Solid line*, Control; *dashed line*, cocaine.

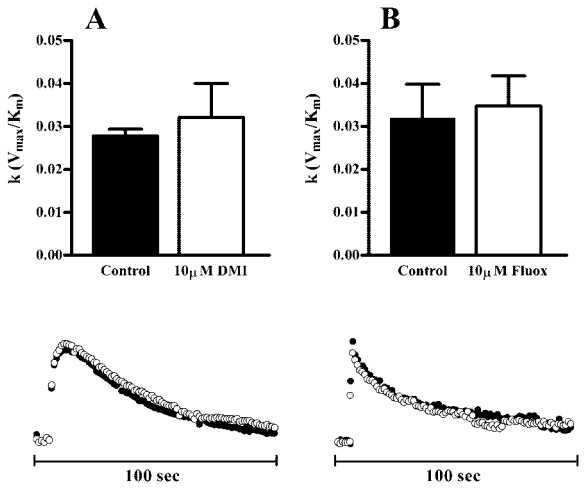

Figure 3. Lack of effect of desipramine and fluoxetine on DA clearance in the slices from NAc shell of DAT-KO mice. *A*, *B*, *Top*, The rate of DA clearance, reported as a rate constant *k* before and after drug administration. Desipramine (*A*) and fluoxetine (*B*) had no effect on DA clearance in the NAc shell of DAT-KO animals ($p > 0.05$). *Filled bars*, Control ($n = 5–6$); *open bars*, 20 min application of 10 μM drug ($n = 5–6$). *A*, *B*, *Bottom*, DA efflux in response to single electrical pulses in a single shell NAc slice. Control curves are *filled circles*; curves with desipramine (10 μM) (*A*) and fluoxetine (10 μM) (*B*) are *open circles*. Data are plotted every 10th point for visual clarity.

DISCUSSION

Microdialysis measurements by Carboni et al. (2001) found cocaine-induced elevations in DA in the NAc of DAT-KO mice and postulated that cocaine inhibition of the NET was responsible. Microdialysis and voltammetry are complementary methods measuring different aspects of DA neurotransmission (Westerink and Justice, 1991; Jones et al., 1999; Budygin et al., 2000). Microdialysis provides information on changes in basal extracellular DA levels that are regulated by multiple mechanisms, including release, uptake, synthesis, and metabolism. In contrast, FSCV does not measure basal DA levels, but the high temporal and spatial resolution of this technique allows evaluation of drug effects on the dynamics of DA clearance and evoked DA release (Westerink and Justice, 1991; Jones et al., 1999). In the present study, FSCV was used to test the hypothesis that cocaine alters DA clearance in the NAc of DAT-KO mice.

We report here that in agreement with previous *in vivo* and *in vitro* studies (Jones et al., 1998; Benoit-Marand et al., 2000), DA clearance was dramatically prolonged in DAT-KO compared with wild-type mice. No differences were observed in the kinetics of DA elimination between the shell and core of the NAc in DAT-KO mice. Consistent with earlier studies in rats (Jones et al., 1996), DA uptake was slower in the shell than in the core of the NAc in wild-type mice. Cocaine failed to change DA clearance or evoked release in both regions of the NAc of DAT-KO mice. However, in wild-type mice, cocaine was effective in decreasing the rate of DA clearance in all brain regions tested.

Several investigations have demonstrated that in wild-type mice the NET does not contribute to DA uptake in the NAc (Tanda et al., 1997; Carboni et al., 2001; Lee et al., 2001). However, it was suggested (Carboni et al., 2001) that the deletion of DA uptake in the NAc could lead to alternative clearance via the NET. In fact, in the prefrontal cortex, where NE innervation prevails over DA innervation, the NET is capable of maintaining "normal" rates of uptake in DAT-KO mice (Mundorf et al., 2001). In contrast to our expectation, no evidence of alternative uptake was found in the NAc of the DAT-KO mice. First, the clearance rate of DA in the NAc shell, where a greater NE innervation is found (Berridge et al., 1997; Delfs et al., 1998) and alternative clearance is most likely to take place, was identical to that of the core NAc and dorsal striatum (Jones et al., 1998; Benoit-Marand et al., 2000). Second, desipramine, a potent NET inhibitor, was not able to change DA clearance in the shell of the NAc of DAT-KO mice, similar to findings in wild-type mice. Therefore, the present findings exclude the possibility that after the genetic deletion of the DAT, the NET actively clears DA in the NAc. Because identical results were obtained with fluoxetine, a SERT inhibitor, alternative DA clearance via SERT is also unlikely. This is in agreement with the fact that cocaine, which inhibits transport at DAT, NET, and SERT (Ritz et al., 1990), was ineffective in inhibiting DA clearance in the shell and core of the NAc in DAT-KO mice. It is possible that NET or SERT in the NAc shell may provide a minor clearance mechanism for DA that is masked by diffusion in the DAT-KO mice. However, our findings rule out the involvement of these monoamine transporters in the cocaine-induced increase of DA (Carboni et al., 2001) because the increase in extracellular DA in DAT-KO mice is large (Carboni et al., 2001) and would be readily detectable by voltammetry. Therefore, we suggest that the effect of cocaine in DAT-KO mice is not caused by inhibition of DA clearance in the NAc.

For DA levels to be increased by cocaine administration as shown (Carboni et al., 2001), either uptake or release could be altered. Elevations in impulse-dependent release of DA might contribute to the increase in DA levels within the NAc (Grace, 2000). Cocaine did not change DA release under the present experimental conditions; however, because the cell bodies of DA neurons are removed in our preparations, we evaluated the effect of cocaine on DA release only at the presynaptical terminal. Therefore, we propose that the mechanism of cocaine interaction with DA neurotransmission does not take place at the level of presynaptic terminals in the DAT-KO mice, but cell body regions may be involved in the cocaine-induced DA increase in the NAc. This suggests that these brain areas may play an important role in cocaine reinforcement in DAT-KO mice. Further studies are necessary, however, to ascertain how cocaine interacts with the DA system in DAT-KO mice.

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