The Dopamine D3/D2 Agonist (+)-PD-128,907 [(R-)(+)-trans-3,4a,10b-Tetrahydro-4-propyl-2H,5H-[1]benzopyrano[4,3-b]-1,4-oxazin-9-ol)] Protects against Acute and Cocaine-Kindled Seizures in Mice: Further Evidence for the Involvement of D3 Receptors

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ABSTRACT

Previous findings have demonstrated a protective role for dopamine D3/D2 receptor agonists in the convulsant and lethal effects of acutely administered cocaine. Data are provided here to establish that the protection occurs through a D3-linked mechanism and that protection is extended to seizure kindling. The D3 antagonist SB-277011-A [4-quinolinecarboxamide, N-[trans-4-[2-(6-cyano-3,4-dihydro-2(1H)-isooquinolínio)ethyl]- cyclohexyl][9CI]] prevented the anticonvulsant effect of the D3/D2 receptor agonist (+)-PD-128,907 [(R-)(+)-trans-3,4a,10b-tetrahydro-4-propyl-2H,5H-[1]benzopyrano[4,3-b]-1,4-oxazin-9-ol)] on cocaine-induced seizures. The protection afforded by the D3/D2 agonist, (+)-PD-128,907, was eliminated in D3 receptor-deficient mice. In D3 receptor knockout mice, the anticonvulsant effects of (+)-PD-128,907 were preserved. (+)-PD-128,907 also prevented the acquisition and expression of cocaine-kindled seizures engendered by repeated daily dosing with 60 mg/kg cocaine. (+)-PD-128,907 also blocked the seizures induced in mice fully seizure kindled to cocaine. Although repeated dosing with cocaine increased the potency of cocaine to produce seizures and lethality (decreased ED50 values), daily coadministration of (+)-PD-128,907 significantly prevented this potency shift. In mice treated daily with cocaine and (+)-PD-128,907, the density, but not the affinity, of D3 receptors was increased. The specificity with which (+)-PD-128,907 acts upon this cocaine-driven process was demonstrated by the lack of a significant effect of (+)-PD-128,907 on seizure kindling to a GABA_A receptor antagonist, pentylenetetrazol. Taken together and with literature findings, the data indicate that dopamine D3 receptors function in the initiation of a dampening mechanism against the toxic effects of cocaine, a finding that might have relevance to psychiatric disorders of drug dependence, schizophrenia, and bipolar depression.

The widespread abuse of cocaine has been associated with a host of medical complications (Substance Abuse and Mental Health Services Administration, Office of Applied Studies, 2003). Of the increasing numbers of emergency medical department episodes, 12% of patients have required anticonvulsant therapy (Derlet and Albertson, 1989; Dhuna et al., 2003). However, some seizures and status epilepticus from cocaine are resistant to standard therapies and can be fatal (Dhuna, 1991). The D3 dopamine receptor, a member of the family of D2-like dopamine receptors, was cloned in 1990 (Sokoloff et al., 1990). Expressed in roughly 10-fold lower density than D2 receptors, D3 receptors have been of interest because of their preferential localization in the nucleus accumbens, a brain area involved in the reinforcement of behavior (for review, see Levant, 1997). Consistent with this pattern of expression, the D3 receptor seems to be involved in mediating effects of psychostimulants. It is noteworthy that (+)-PD-128,907 and other D3/D2 agonists decrease cocaine self-administration in self-administration in

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ABBREVIATIONS: (+)-PD-128,907, (R-)(+)-trans-3,4a,10b-tetrahydro-4-propyl-2H,5H-[1]benzopyrano[4,3-b]-1,4-oxazin-9-ol]; PD 58491, (3-[4-(2-[4-(3-diethylamino-propoxy)-phenyl]-benzoimidazol-2-yl)-butyl]-1H-benzoimidazol-2-yl]-phenoxy)-propyl]-diethyl-amine; KO, knockout; WT, wild type; PTZ, pentylenetetrazol; SB-277011-A, 4-quinolinecarboxamide,N-[trans-4-[2-(6-cyano-3,4-dihydro-2(1H)-isooquinolínio)ethyl]-cyclohexyl][9CI]; ANOVA, analysis of variance.

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animals, suggesting that the receptor plays a role in the reinforcing effects of cocaine (Caine and Koob, 1993; Pilla et al., 1999; Vorel et al., 2002). Pharmacological and behavioral evidence has also linked agonist activity at D₃ receptors to yawning in rats (Collins et al., 2005, 2007).

D₃ receptors might also be modulators of cocaine toxicity. D₂/D₃ receptor agonists, including (+)-PD-128,907, prevent the seizures and lethality produced by cocaine (Witkin et al., 2004). (+)-PD-128,907 blocked cocaine-induced seizures in mice under conditions in which, as in the clinical setting, they are relatively resistant to standard anticonvulsant therapy (see Witkin and Tortella, 1991; Gasior et al., 1997; Witkin et al., 1999). In contrast, (+)-PD-128,907 was devoid of protective efficacy against a broad range of other nondopaminergic convulsants, documenting the selectivity of its effects for cocaine-induced seizures. This protection was species-specific and reversible by an antagonist of D₃ receptors (PD 58491). Furthermore, the anticonvulsant potencies of D₃/D₂ agonists were positively associated with potencies in a functional assay of D₃ but not D₂ receptor function, suggesting a critical and dominant role for D₃ receptors in the antagonism of cocaine-induced seizures.

However, neither D₃ agonist nor antagonist tools are sufficiently selective for D₃ receptors in vivo; therefore, proof for a role of D₃ receptors in cocaine toxicity must come from convergent data. In the present report, we present evidence from D₃ and D₂ receptor knockout mice, confirming previous pharmacological data, that support a role for D₃ receptors in the anticonvulsant effects of (+)-PD-128,907. Such evidence is critical in light of the discrepancies in the literature from pharmacological and transgenic technologies in the area of D₃ receptors. For example, although agonists with D₃ receptor affinity decrease body temperature in rats, this effect is also engendered in D₃ receptor-deficient mice (Xu et al., 1999; Perachon et al., 2000). In addition, the observations with acutely administered cocaine (Witkin et al., 2004) were extended in the present report to sensitization to the convulsant effects of cocaine (kindled seizures).

**Materials and Methods**

**Animals.** The following mice were used: experimentally naive, male Swiss-Webster mice (29–37 g) (Taconic Farms, Germantown, NY), D₃ receptor-deficient mice (The Jackson Laboratory, Bar Harbor, ME) (32–42 g), and D₃ receptor-deficient mice (26–34 g). D₃ receptor knockout (KO) mice were derived from the original mice of Accili et al. (1996) and bred homozygous to homozygous after initial breeding to a congenic line. The D₃ receptor KO mice were derived from the original mice of Low and colleagues (B6.Cg-Drdrl₃m1Low mutant mice) (Kelly et al., 1998) and were re-derived on a C57BL/6 background at Taconic Farms and bred to a congenic line; the mice are used here with acknowledgment of this research group. The wild-type (WT) control mice for the D₃ and D₃ receptor KO mice were C57BL/6J mice. Because both strains were congenic lines, this strain was used as the WT control. Mice were bred at the same breeder, age-matched (~4–6 weeks old), shipped simultaneously, and housed identically.

Mice were group housed in a temperature-controlled vivarium. For acute experiments, mice were used only once. For kindling experiments, the mice were used repeatedly for the duration of the study. All animals were acclimated to their home cages and to the light/dark cycle (lights on at 6:00 AM for 12 h) for at least 5 days before testing. Food and water were continuously available in their living cages. At least six mice per group were used in the in vivo experiments. Animals used in the present study were maintained in facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care. All experimentation was conducted in accordance with the guidelines issued by the Institutional Care and Use Committees and included in the Guide for Care and Use of Laboratory Animals (National Research Council, 1996).

**Cocaine-Induced Seizures.** Acute experiments were conducted in which mice received injections with vehicle or (+)-PD-128,907 s.c., 30 min before cocaine (75 mg/kg i.p.), and then placed in individual Plexiglas containers (14 x 25 x 30 cm high) for observation. Cocaine-induced convulsions were defined as loss of the righting response for at least 5 s and the occurrence of clonic limb movements. Tonic seizures and death were rarely observed. The animals were observed for 30 min post-cocaine administration for the presence of seizures and then at 60 min for any potential lethality. Dopamine D₁ and D₃ receptor KO mice were compared with WT control mice to determine the following: (1) whether the potency of cocaine to induce seizures differed in the receptor KO lines, and (2) whether the anticonvulsant effects of (+)-PD-128,907 were affected by the gene deletions. In addition, Swiss-Webster mice were studied in parallel experiments in which the ability of a D₃ receptor antagonist, SB 27011-A (Reavell et al., 2000), to augment or prevent the convulsant effects of cocaine was explored.

**Cocaine Seizure Kindling.** Cocaine-kindled seizures were produced by administration of 60 mg/kg cocaine i.p. on five successive days (Miller et al., 2000). The ability of (+)-PD-128,907 to prevent the acquisition of kindling was tested by pretreating mice with (+)-PD-128,907 (0.3–3 mg/kg s.c.) 30 min before each cocaine administration. Blockade of kindling development was assessed by administration of a challenge dose of cocaine alone on day 6 after five prior treatments of combinations of (+)-PD-128,907 + cocaine or saline + cocaine. Additional experiments were also conducted in which animals previously kindled to cocaine for 5 days were tested to determine whether (+)-PD-128,907 would prevent seizures in these fully seizure kindled mice on day 6. Other experiments evaluated whether cotreatment with (+)-PD-128,907 and cocaine would alter the potency to induce seizures. Separate groups of mice kindled with cocaine for 5 days either alone or in the presence of (+)-PD-128,907 were tested on day 6 with a range of doses of cocaine alone to establish dose-effect curves. Finally, to establish whether prior exposure to (+)-PD-128,907 alone could alter the convulsant effects of cocaine, (+)-PD-128,907 was administered for five consecutive days; on day 6, mice were challenged with cocaine alone. Observation periods were as in the acute experiments described above.

**D₃ Receptor Binding.** Independent cocaine kindling experiments were carried out with the purpose of defining D₃ receptor changes that might have been generated by repeated cocaine dosing under these conditions and whether coadministration of (+)-PD-128,907 altered any such changes engendered. In these experiments, mice were given one of four treatments for 5 days and sacrificed by decapitation 24 h after the final treatment. Treatment groups were as follows: 1) saline + saline; 2) saline + cocaine (60 mg/kg); 3) saline + (+)-PD-128,907 (3 mg/kg); and 4) (+)-PD-128,907 (3 mg/kg) + cocaine (60 mg/kg). The density and affinity of D₃ dopamine receptors were assessed using [³H]-PD-128,907 as described previously (Bancroft et al., 1998). Ventral striata (nucleus accumbens and olfactory tubercle) was dissected on ice from each brain (20–30 brains per treatment group). Pooled tissue was homogenized with a PRO250 homogenizer (setting 4 of 6) (PRO Scientific, Monroe, CT) for 10 s in 20 volumes of assay buffer (50 mM Tris, 1 mM EDTA, pH 7.4, at 23°C). The crude homogenate was centrifuged twice at 48,000g for 15 min, resuspending the pellet in 20 volumes of assay buffer each time. The final pellet from membrane preparation was resuspended in buffer to yield a final concentration of 8.5 mg of original wet weight/ml. Binding assays were performed in duplicate in disposable polystyrene tubes. The final assay volume was 0.5 ml. For saturation analyses, 6 or 8 concentrations of (+)-[N-propyl-2,3-³H]PD-128,907 (114 Ci/mmol; Amersham, Arlington Heights, IL, now GE Health-
seizures can be quantified with PTZ seizures, a seizure scoring
analysis was limited to two independent determinations of [3H]PD-128907 binding with each pooled tissue sample. Ventral striata (nucleus accumbens and olfactory tubercle) were analyzed.

Compounds for in Vivo Pharmacology. (+)-PD-128,907, PTZ, and cocaine HCl were obtained from Sigma-Aldrich (St. Louis, MO). SB-27011-A was synthesized at Eli Lilly and Company. (+)-PD 126,907, PTZ, and cocaine were dissolved in distilled water or physiological saline. SB-277011-A was dissolved in 20% β-cyclodextrin. (+)-PD-128,907 and SB-277011-A were administered by s.c. injection, 30 min before saline or cocaine. Cocaine and PTZ were given i.p.

Analysis of Seizure Data. Quantal seizure data were evaluated with Fisher's exact probability test. The continuous data from seizure severity rating scales was subjected to analysis of variance (ANOVA) followed by post hoc Dunnett's tests. Dose-effect curves were analyzed by ANOVA, and linear regression analyses were performed to determine ED_{50} values and 95% confidence limits. D_{3} receptor binding data were analyzed using the nonlinear least-squares curve-fitting program LIGAND.

Results

Effect of D_{3} or D_{2} Receptor Deletion on the Ability of Cocaine to Induce Convulsions. Cocaine produced dose-dependent increases in clonic convulsions in mice. There were no statistical differences in the dose-effect functions in WT and D_{3} receptor KO mice (Fig. 1). The ED_{50} for convulsions was 76.6 (95% confidence limit, 71.7–81.4) mg/kg in WT mice and 80.8 (72.6–88.9) mg/kg in the knockout mice. There was no significant difference between these values as assessed by parallel line bioassay. Although constitutive deletion of the D_{3} receptor did not alter the convulsant effects of acutely administered cocaine, the anticonvulsant effects of (+)-PD-128,907 (3 mg/kg) were absent in mice devoid of D_{3} receptors (Fig. 2). In contrast to the results in mice with the D_{3} receptor protein deleted, D_{2} receptor KO mice, like WT controls, had an intact effect of the anticonvulsant effect of (+)-PD-128,907 (Fig. 2). As with the D_{3} receptor KO mice, D_{2}

Fig. 1. No significant differences in cocaine-induced convulsions in wild-type and D_{3} receptor-deficient mice. Acute doses of cocaine (i.p.) produced dose-dependent increases in clonic convulsions. Each point represents the percentage of mice convulsing at each dose (n = 8 mice/group).

care, Piscataway, NJ) were used (0.1–3 nM). Binding was initiated by the addition of membrane homogenate. Nonspecific binding was defined by 1 μM spiperone. Assay tubes were incubated for 3 h at 23°C. The reaction was terminated by rapid filtration through Whatman GF/B filters (Whatman, Clifton, NJ; now GE Healthcare, Piscataway, NJ) pretreated with 0.5% polyethylenimine using a Brandel cell harvester (Brandel Inc., Gaithersburg, MD). Filters were washed three times with 3 ml of ice-cold buffer (50 mM Tris, pH 7.4 at 23°C), and placed in scintillation vials. After the addition of Beckman Ready Protein- scintillation cocktail, vials were shaken, allowed to equilibrate for 2 h, and radioactivity quantitated using a Beckman 6500 scintillation counter (Beckman Coulter, Fullerton, CA). Protein concentrations were determined using the BCA method (Pierce Chemical, Rockford, IL). Specific binding of [3H]PD-128907 is expressed as femtomoles per milligram of protein. Because of the limited amount of ventral striatal tissue available per mouse, analysis was limited to two independent determinations of [3H]PD-128907 binding with each pooled tissue sample. Ventral striata (nucleus accumbens and olfactory tubercle) were analyzed.

Pentylenetetrazol Seizure Kindling. Swiss-Webster mice were given a regimen of pentylenetetrazol (PTZ) that engenders seizure kindling as described previously (Gasior et al., 1998). Under this procedure, mice were given i.p. injections of 45 mg/kg PTZ on days 1, 3, 5, and 8. The percentage of mice convulsing was determined over a 30-min period post-PTZ administration. Because the severity of seizures can be quantified with PTZ seizures, a seizure scoring system modified from Lösch et al. (1991) was used in addition to assessing the percentage of mice exhibiting seizures, as with cocaine. Each mouse was rated during the observation period with the highest seizure score observed for that mouse. The rating system scored mice as follows: 1 for one or more generalized myoclonic twitches of the whole body; 2 for repeated clonic seizures of fore- and/or hindlimbs, without loss of righting response; 3 for a generalized clonic seizure of fore- and hindlimbs, during which the mice demonstrated loss of righting; 4 for loss of righting followed by a tonic hindlimb seizure; and 5 for loss of righting with tonic fore- and hindlimb seizure followed by death. From these scores, means ± S.E.M. for each day of kindling were computed.

Fig. 2. Protective effects of (+)-PD-128,907 against cocaine seizures are absent in D_{3} receptor-deficient mice but spared in D_{2} receptor knockout mice. Unfilled bars, effects of cocaine + saline; filled bars, effects of cocaine with 3 mg/kg (+)-PD-128,907. * = significant efficacy as an anticonvulsant (Fisher's exact probability test, p < 0.05). Each bar represents the percentage of mice convulsing at each dose (n = 8–12 mice/group).
receptor KO mice did not significantly differ from the WT mice in basal seizure level induced by this dose of cocaine (Fig. 2).

**Effect of D₃ Receptor Blockade on the Ability of Cocaine to Induce Convulsions.** As with receptor deletion (Fig. 1), pharmacological blockade of D₃ receptors in Swiss-Webster mice, achieved by administration of the D₃ receptor antagonist SB-277011-A, did not enhance or prevent the convulsant effects of cocaine (Table 1). Nonetheless, SB-277011-A prevented the anticonvulsant effects of (+)-PD-128,907 in Swiss-Webster mice. In this latter experiment, mice were given cocaine alone, cocaine with (+)-PD-128,907, or SB-277011-A before cocaine (+)-PD-128,907 (Fig. 3). The attenuation of the convulsant effect of cocaine by (+)-PD-128,907 was prevented by the D₃ receptor antagonist.

**Sensitization to the Convulsant Effects of Cocaine.** Daily administration of 60 mg/kg cocaine produced progressive increases in the percentage of mice exhibiting convulsions from 19 to 90% over the 5 days of treatment, with cumulative lethality occurring in 26% of the mice at the end of kindling (Fig. 4, filled circles). In contrast, in the presence of (+)-PD-128,907 (3 mg/kg), cocaine produced a maximum of only 12% (triangles, top) convulsions and a cumulative lethality on the order of 15% (triangles, bottom). Protection against the acquisition of cocaine seizures was also conferred by a 10-fold lower dose of (+)-PD-128,907, 0.3 mg/kg (squares, top), but this dose was insufficient to protect against the lethal consequences of cocaine kindling (squares, bottom).

Although these data document the ability of (+)-PD-128,907 to block the acquisition of cocaine-kindled seizures and lethality, they do not address whether this compound can prevent the development of the epileptogenic process resulting from repeated cocaine exposure. Due to its acute anticonvulsant effects against cocaine, (+)-PD-128,907 might simply be masking the convulsant effects of cocaine. Evaluation of effects of (+)-PD-128,907 on seizure development was accomplished by giving a challenge dose of cocaine alone on day 6 after mice had been treated with combinations of cocaine plus (+)-PD-128,907 on days 1 to 5. Results indicated that prior coadministration of (+)-PD-128,907 with cocaine produced a dose-dependent suppression of the development of kindled seizures (Fig. 5). Administration of (+)-PD-128,907 also prevented the increased seizure incidence produced in mice previously kindled to cocaine by repeated dosing with cocaine alone (Fig. 6).

In cocaine-kindled mice, the ED₅₀ for cocaine convulsions was decreased as previously reported (see Miller et al., 2000) (Fig. 7, compare bars A and B). This increase in the potency of cocaine to engender convulsions was prevented by the coadministration of (+)-PD-128,907 with cocaine for five consecutive days. ED₅₀ values for cocaine alone determined on day 6 were significantly different in mice given (+)-PD-128,907 + cocaine versus mice given cocaine alone for the 5 preceding days (Fig. 7, compare bars B and C). In addition, the potency of cocaine to produce lethality was reduced by the coadministration of cocaine and (+)-PD-128,907. In this experiment, mice were kindled with 60 mg/kg cocaine + saline or 60 mg/kg cocaine + 3 mg/kg (+)-PD-128,907 for 5 days. On day 6, mice were tested with 85 mg/kg cocaine alone. When challenged with 85 mg/kg cocaine on day 6, 80% of mice kindled in the absence of (+)-PD-128,907 died, whereas only 25% mice kindled with cocaine in the presence of (+)-PD-128,907 died (p < 0.05). Dose-effect curves were not collected in the lethality experiment to reduce the number of mice subjected to this procedure.

It is possible that prior administration of (+)-PD-128,907 alone for 5 days could decrease the sensitivity of mice to the convulsant and lethal effects of cocaine. That is, (+)-PD-128,907 may have increased the threshold of cocaine. To evaluate this possibility, 3 mg/kg (+)-PD-128,907 was given alone for five consecutive days. Then, on day 6, separate groups of mice were challenged with cocaine alone. The convulsant effects of cocaine were not significantly altered by prior dosing for 5 days with (+)-PD-128,907 alone; challenge with 60 mg/kg cocaine on day 6 generated convulsions in one of eight mice, regardless of whether (+)-PD-128,907 was given for 5 days previously. Likewise, when challenged with 75 mg/kg cocaine on day 6, seven of eight mice exhibited convulsions when vehicle had been given for 5 previous days; six of eight exhibited convulsions on day 6 after a 5-day regimen of (+)-PD-128,907. These findings indicate that the presence of (+)-PD-128,907 alone was not responsible for changes in the convulsant threshold of cocaine.

**Sensitization to the Convulsant Effects of Pentylenetetrazol.** To determine whether the effects of (+)-PD-128,907 on cocaine-kindled seizures generalize to another

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**TABLE 1**

The D₃ receptor antagonist (SB-277011-A) did not alter the convulsant effects of cocaine

<table>
<thead>
<tr>
<th>SB-277011-A</th>
<th>45 mg/kg Cocaine</th>
<th>60 mg/kg Cocaine</th>
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</thead>
<tbody>
<tr>
<td>0 mg/kg</td>
<td>1/12</td>
<td>5/12</td>
</tr>
<tr>
<td>3 mg/kg</td>
<td>1/6</td>
<td>4/6</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>0/6</td>
<td>4/6</td>
</tr>
<tr>
<td>30 mg/kg</td>
<td>0/6</td>
<td>3/6</td>
</tr>
</tbody>
</table>

Vehicle or SB-277011A was administered s.c., 30 min before cocaine challenge (i.p.). The proportion of mice exhibiting convulsions is shown. Effects of cocaine alone were compared with effects of cocaine in the presence of SB-277011A with Fisher’s exact probability test without any significant differences revealed.
convulsant agent, the effects of (+)-PD-128,907 were examined in the PTZ-kindled seizure model. PTZ (45 mg/kg i.p.) administered every other day resulted in successive increases in the percentage of mice exhibiting convulsions and in the severity of the seizures (Fig. 8, left). When PTZ was given every other day in the presence of 3 mg/kg (+)-PD-128,907, there were similar increases in both measures of seizure outcome, and there were no statistical differences in the values from mice given PTZ alone or PTZ in the presence of (+)-PD-128,907 (Fig. 8, left). The increase in seizure incidence and severity were statistically significant on the fifth injection of PTZ regardless of whether (+)-PD-128,907 was coadministered. For seizure severity scoring, a two-way ANOVA documented the significant increase observed over repeated treatments ($F_{3,132} = 5.3$, $p < 0.005$), whereas there was not a significant effect of treatment with (+)-PD-128,907 ($F_{1,132} = 4.2$, $p > 0.05$), although a trend was observed. On day 10, mice in both the PTZ alone group and PTZ + PD-128,907 group were given 45 mg/kg PTZ alone to determine the influence of (+)-PD-128,907 on the kindling process. As with the acquisition of PTZ kindling, there were no significant differences in the effects of PTZ alone on day 10 that depended on the prior drug treatments (Fig. 8, right).

**D$_3$ Receptor Alterations with Repeated Drug Treatments.** The ability of cocaine or cocaine + PD128,907 to modify the density or binding affinity of D$_3$ receptors in ventral striatal (nucleus accumbens and olfactory tubercle) membranes was assessed after administration for five consecutive days as in the kindling experiments described above. Separate groups of mice were given vehicle + vehicle, vehicle + cocaine (60 mg/kg), (+)-PD-128,907 (3 mg/kg) + vehicle, or (+)-PD-128,907 (3 mg/kg) + cocaine (60 mg/kg). $K_a$ values did not differ significantly

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**Fig. 4.** (+)-PD-128,907 protected against the acquisition of cocaine-kindled seizures (top) and the death associated with cocaine kindling (bottom). Mice were given 60 mg/kg cocaine daily for five consecutive days with saline (filled circles), with 0.3 mg/kg (+)-PD-128,907 (squares), or with 3 mg/kg (+)-PD-128,907 (triangles). *, significant differences from the effects observed on day 1 (Fisher’s exact probability test, $p < 0.05$). †, significant difference from effects observed in the control group (cocaine + saline) on the same experimental session. Each point represents the percentage of mice convulsing at each dose ($n = 10$ mice/group).

**Fig. 5.** (+)-PD-128,907 protected against the development of cocaine-kindled seizures. Mice were given 60 mg/kg cocaine daily for five consecutive days with saline (filled circles), with 0.3 mg/kg (+)-PD-128,907 (squares), or with 3 mg/kg (+)-PD-128,907. Shown are data on the percentage of mice exhibiting seizures on day 6 in the presence of 60 mg/kg cocaine alone. *, significant protection against kindling compared with the (+)-PD-128,907-null group (0 mg/kg) (Fisher’s exact probability test, $p < 0.05$).

**Fig. 6.** (+)-PD-128,907 protected against the convulsant effects of cocaine in mice previously kindled to cocaine seizures with repeated dosing. Five days of prior cocaine administration (60 mg/kg i.p.) produced an increase in seizure incidence as indicated by *(Fisher’s exact probability test, $p < 0.05$). **, significant difference from effects observed in the control group (cocaine + saline) on the same experimental session. Each point represents the percentage of mice convulsing at each dose ($n = 10$ mice/group).
D3/D2 receptor agonists to protect against the acute convulsant effects of cocaine were also significantly dampened by a D3 receptor-relevant dose of (+)-PD-128,907 (Fig. 4). Furthermore, despite the in vivo impairment of (+)-PD-128,907 upon D2 receptors at higher doses, its anticonvulsant effects were prevented in D3 receptor-deficient mice, but not in mice without D2 receptors (Fig. 2). In addition, the potency of a series of agonists to attenuate cocaine-induced seizures was positively associated with functional activity at D3 but not D2 receptors (Witkin et al., 2004; Perachon et al., 2000; Chaperon et al., 2003). Thus, the present findings with transgenic mice taken in conjunction with the pharmacological data (Witkin et al., 2004) provide the first consistent linkage of D3 receptor function to in vivo activity.

**D3 Receptors May Play a Regulatory Process in the Convulsant Effects of Cocaine.** Are actions of dopamine at D3 receptors causal in generating cocaine seizures, or do they only serve a regulatory role once initiated? If increased stimulation of D3 receptors, secondary to the blockade of dopamine uptake by cocaine, is responsible for the initiation of seizures, cocaine would be anticipated to exhibit decreased seizurogenic potency in mice treated with a D3 antagonist or in mice without D3 receptors. Mice lacking D3 receptors did not differ from intact mice in the convulsant effects of cocaine per se, nor did the addition of a D3 receptor antagonist modify the convulsant effects of cocaine. Together, these findings demonstrate that the induction of seizures by cocaine is not mediated by stimulation of D3 receptors and thus most likely occurs through a non-D3-driven mechanism (e.g., glutamate release) (Reid et al., 1997; Robinson et al., 1997; Witkin et al., 1999; D3 receptors, on the other hand, could provide one braking mechanism postsiezure initiation.

**D3 Receptors Control Cocaine Seizure Kindling.** Subconvulsant doses of cocaine when given repeatedly can ultimately result in full-blown convulsions and death (Post and Weiss, 1989; Miller et al., 2000). This process of seizure sensitization or kindling also occurs with other convulsant stimuli and has been used to model the progressive, epileptogenic process of human epilepsies and pathogenesis of refractory epilepsy in humans (Löschel and Schmidt, 1988;
The present study demonstrated protective efficacy of (+)-PD-128,907 against cocaine-kindled seizures. This is the first report demonstrating the efficacy and specificity of a dopamine agonist against different phases of a seizure kindling process. Furthermore, pharmacological evidence is brought to bear in support of the idea that, like acute cocaine-induced convulsions (Witkin et al., 2004), the increasing toxicity of repeated cocaine administration can also be regulated by D₃ receptor-associated mechanisms.

Coadministration of (+)-PD-128,907 with cocaine over as short a time as five doses (effective in producing seizure kindling) resulted in almost a 50% increase in D₃ receptor density without affecting affinity, and there was also a trend toward an increase in the density of receptors after the short-term administration of cocaine alone. The increase in D₃ receptors observed here and in the postmortem findings of Staley and Mash (1996) might represent an adaptive process compensating for increased dopaminergic tone. Increases in D₃ receptors have also been observed in a number of reports in which other compounds that raise central dopamine levels (e.g., levodopa, nicotine) have been repeatedly administered (Bordet et al., 1997; Le Foll et al., 2003a), but this effect has not always been observed (see Chiang et al., 2003; Richtand et al., 2003).

**TABLE 2**

Affinity and density of D₃ receptors after experimental treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Kᵩ</th>
<th>Bₘₐₓ</th>
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<tbody>
<tr>
<td>Saline-saline</td>
<td>0.65</td>
<td>18.6</td>
</tr>
<tr>
<td>Saline-cocaine</td>
<td>0.64</td>
<td>24.0</td>
</tr>
<tr>
<td>(+)-PD-128,907-saline</td>
<td>1.4</td>
<td>16.9</td>
</tr>
<tr>
<td>(+)-PD-128,907-cocaine</td>
<td>0.63</td>
<td>30.9*</td>
</tr>
<tr>
<td>Acute cocaine</td>
<td>0.48</td>
<td>22.0</td>
</tr>
</tbody>
</table>

* Effects significantly different from saline-saline treatment effects.

McNamara et al., 1993). The present study demonstrated protective efficacy of (+)-PD-128,907 against cocaine-kindled seizures. This is the first report demonstrating the efficacy and specificity of a dopamine agonist against different phases of a seizure kindling process. Furthermore, pharmacological evidence is brought to bear in support of the idea that, like acute cocaine-induced convulsions (Witkin et al., 2004), the increasing toxicity of repeated cocaine administration can also be regulated by D₃ receptor-associated mechanisms.

Coadministration of (+)-PD-128,907 with cocaine over as short a time as five doses (effective in producing seizure kindling) resulted in almost a 50% increase in D₃ receptor density without affecting affinity, and there was also a trend toward an increase in the density of receptors after the short-term administration of cocaine alone. The increase in D₃ receptors observed here and in the postmortem findings of Staley and Mash (1996) might represent an adaptive process compensating for increased dopaminergic tone. Increases in D₃ receptors have also been observed in a number of reports in which other compounds that raise central dopamine levels (e.g., levodopa, nicotine) have been repeatedly administered (Bordet et al., 1997; Le Foll et al., 2003a), but this effect has not always been observed (see Chiang et al., 2003; Richtand et al., 2003).

**Regulation of Dopamine Kinetics Might Be One Mechanism Associated with the Anticonvulsant Activity of Dopamine D₃ Receptor Agonsists.** If, as the data presented indicate, (+)-PD-128,907 produces its anticonvulsant effects through its actions at D₃ receptors, the increase in D₃ density produced by treatment of cocaine with (+)-PD-128,907 could facilitate this process. Increases in receptor density would enhance D₃-mediated uptake of dopamine (see Witkin et al., 2004), a mechanism that was demonstrated to occur with this ligand through a D₃-mediated mechanism.
across some of its dose range (Zapata and Shippenberg, 2002). This, in turn, could disrupt dopamine-mediated processes that might be involved in the induction of seizures and might account for the ability of (+)-PD-128,907 to attenuate cocaine kindling. At present, such a possibility is only indirectly supported by the data. D3 receptor agonists can modify dopamine transport kinetics and number that could feed back to regulate dopamine uptake (see Zapata and Shippenberg, 2002), all of which control cocaine toxicity. A comparable line of evidence and argument has been brought to bear regarding dopaminergic neurotoxicity as it pertains to the protective effects of the dopamine D2/D3 receptor agonist pramipexole, an effect mediated through enhancement of D3-mediated uptake processing (see Joyce et al., 2004; Joyce and Millan, 2007). Recent experiments in cell lines expressing D3 receptors and the dopamine transporter provide evidence that agonist binding to D3 receptors increases the activity of the dopamine transporter and alters cell surface expression, suggesting that D3 receptor modulators can interfere with the trafficking and activity of the dopamine transporter (Zapata et al., 2007).

Potential Implications for Psychiatric Disease. A convergence of data has generated a structural framework to account for the genesis of schizophrenic symptomatology (see Lieberman et al., 1997). The ventral striatum is highly enriched in D3 receptors (see Levant, 1997), and these D3 receptor-containing neurons have been shown to be a potential target of the activity of atypical antipsychotic drugs (Guo et al., 1998; Robertson et al., 2004). Cocaine kindling represents a preclinical model of this sensitization-like process. As with other dopaminergic sensitizing agents (see above), schizophrenia has also been associated with increased dopamine D3 receptors (Gurevich et al., 1997). The ability of dopamine D3 ligands to modulate the kindling process and to modify behavioral sensitization is suggestive of a preclinical foundation for affecting disease progression. (+)-PD-128,907 has been reported to exhibit an atypical antipsychotic profile in one acute model of antipsychotic activity (Witkin et al., 1998). In addition, atypical antipsychotic agents, which all generally have affinity for D3 receptors (Sokoloff et al., 1990), may have disease-modifying influence (see Lieberman et al., 2005). Likewise, cocaine kindling has been used to model bipolar disorder wherein compounds that prevent kindling have been proposed as potential bipolar treatment agents due to the activity of treatment standards (Post and Weiss, 1989). Likewise, dopaminergic sensitization processes are likely to occur during the genesis of drug dependence. Moreover, the central circuitry involved in the pathophysiology of schizophrenia (Lieberman et al., 1997) is similar to that for the development of dependence states (Goldstein and Volkow, 2002). Ligands acting upon dopamine D3 receptors block sensitization to the behavioral activating effects of psychomotor stimulants and to modify behavior in preclinical models of drug dependence (see Bordet et al., 1997; Le Foll et al., 2003b; Richtand et al., 2003; Vorel et al., 2002). The ability of D3 receptor agonists to protect against behavioral sensitization and for D3 receptors to regulate the discriminative and reinforcing effects of cocaine (Caine and Koob, 1993; Acri et al., 1995; Spealman, 1996; Pilla et al., 1999) should continue to keep research focus on dopamine D3 receptors in psychomotor stimulant dependence and associated behavioral and toxic effects.

A Pharmacodynamic Model for D3 Receptor Interaction in Vivo. Definitive evidence that any specific in vivo effect is driven by dopamine D3 receptors has not been readily forthcoming (see Boulay et al., 1999; Xu et al., 1999; Perachon et al., 2000; Chaperon et al., 2003). The present data provide encouragement that cocaine convulsions could serve as a method for identification of the activities of D3 receptor agonists and antagonists in vivo in a preclinical setting in mice. In addition, evidence (unconfirmed with genetic dampening of D3 receptor function) points to the potential use of yawning in rats engendered by D3 agonists as a biomarker of D3 receptor agonism in this species (Collins et al., 2005, 2007).

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References


