channel patches. A patch was considered to contain a single channel when, after ten successive 530-ms applications of 100 μM ACh, no evidence of simultaneous openings by two or more channels was observed. The average peak open probability (P_o) during a 530-ms pulse was 0.6 within the first 5 ms. Thus, if two identical and independent channels were present, the probability of seeing only single-level openings in n consecutive trials is $[2P_o(1 - P_o)]^n$. At the tenth consecutive trial, this probability is 0.00065, which indicates that a single functional channel is present in the patch.

17. A cluster was defined as ten or more consecutive openings separated by closed periods no longer than 20 ms, a criterion previously used to separate gating modes for ACh receptor channels (*6*). This interval yielded the best separation between modes in the distributions of average open times.
18. The maximum-likelihood method was used to fit gamma distributions. Cumulative and probability-density–function distributions were calculated for one, two, or three gamma components according to D. Colquhoun and B. Sakmann, *J. Physiol. London* **369**, 501 (1985). The negative log of likelihood was minimized with the Gauss-Newton method by a routine in the program AJUSTE [O. Alvarez, A. Villarroel, G. Eisenmann, *Methods Enzymol.* **207**, 816 (1992)].
19. A reduction in the mean channel open time and an increase in the number of openings per cluster have been observed after patch excision in the case of embryonic ACh receptors [M. Covarrubias and J. H. Steinbach, *Pfluegers Arch.* **416**, 385 (1990)]. For all clusters of ten or more openings, we measured a significantly ($P < 0.01$) greater number of openings per cluster in the fast mode (20.5 ± 13.85, $n = 130$) than in the slow mode (14.88 ± 8.82, $n = 30$) (*21*). Thus, the excision effects are consistent with a displacement of the receptor kinetics toward the faster gating modes. The excess openings in the fast mode are consistent with the observed low maximal open probability found in response to the 100 μM ACh applications. Thus, the overall open probability results from the balance between the different gating modes (Fig. 3B).
20. D. Shepherd and P. Brehm, unpublished data.
21. For embryonic receptors, we found sixfold differences in the average open times between fast and slow modes, with 2.8 ± 1.8 ms ($n = 130$ clusters) and 17.1 ± 8.4 ms ($n = 30$ clusters), and intracluster open probabilities of 0.57 ± 0.20 and 0.91 ± 0.05, respectively. However, little difference was observed for the average closed durations, with 2.6 ± 2.0 ms and 1.7 ± 0.8 ms for fast and slow modes, respectively. For the adult receptors, the average open time (2.3 ± 1.6 ms), intracluster open probability ($P_o = 0.58 \pm 0.21$), and average closed time (1.7 ± 2.2 ms) measured for the fast mode (49 of 52 clusters) were similar to those measured for the fast mode of the embryonic receptors.
22. In six out of ten patches containing single embryonic receptor channels, the ensembles constructed for records of short average open time indicate a significantly faster current decay than that observed for records of long average open time. However, the data were pooled because there were often too few ensemble averages from individual experiments to provide reliable fits.
23. Because of the infrequent openings to the slow mode in adult receptors, with an 8-ms cutoff, only a small contamination from this mode is expected in the ensembles made from the records of short open time.
24. The estimated steady-state components for the fast mode of both receptor types, 0.09 ± 0.03 for embryonic and 0.05 ± 0.03 for adult, were not significantly different ($P > 0.68$). These values correspond to an average recovery rate of 2.03 ± 1.33 s^{-1} for the fast modes of both embryonic and adult receptors (*26*). This estimate is in better agreement with the fast component of the macroscopic recovery (4.13 ± 2.86 s^{-1}, $P > 0.52$) than the slow component (0.38 ± 0.45 s^{-1}, $P < 0.23$) obtained from the two-pulse protocol (Fig. 1C). This agreement suggests that the rate-limiting step for the recovery from the desensitized state is the transition away from the desensitized state and not the unbinding of the agonist.
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26. The following expression relates open probability to the time constants for macroscopic desensitization (τ): $1/\tau = (1 - P_o)(d^+ + d^-)$. An estimate of d^+ and d^- for each gating mode can be obtained from the fractional current (I) remaining at the end of the response to 530-ms application of the agonist: $I_{end}/I_{peak} \sim d^-/(d^+ + d^-)$.
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Modulation of Cocaine Self-Administration in the Rat Through D-3 Dopamine Receptors

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The reinforcing properties of cocaine are probably mediated by the mesocorticolimbic dopamine pathways in the central nervous system, but not all of the dopamine receptor subtypes involved in cocaine's reinforcing actions have been clearly identified. Recently, the D-3 receptor has been cloned, and its distribution in the brain has been found to be relatively restricted to limbic projections of the midbrain dopamine system. The D-3–selective compounds 7-hydroxy-*N,N*-di-*n*-propyl-2-aminotetralin (7-OHDPAT) and quinpirole potently decreased cocaine self-administration in the rat at doses that were not by themselves reinforcing. Moreover, three dopamine receptor agonists had affinities for binding to the D-3 receptor that correlated highly with their relative potencies in decreasing cocaine self-administration. The D-3 receptor may be involved in the reinforcing effects of cocaine and may be a useful target for the development of new pharmacotherapies for cocaine abuse.

It has been hypothesized that cocaine produces its reinforcing properties by inhibiting dopamine reuptake and thereby potentiating dopaminergic neurotransmission (*1*). Lesion studies have shown that cocaine self-administration in the rat depends on an intact mesocorticolimbic dopamine system (*2*). The recent identification of at least five dopamine receptor subtypes (*3*–*5*) with distinct molecular and pharmacological properties, as well as different anatomical distributions, provides a means for the evaluation of the relative contribution of these subtypes in cocaine reinforcement.

The paucity of highly selective ligands for dopamine receptor subtypes makes the unambiguous determination of the function of these subtypes in cocaine self-administration difficult (*6*). However, the recently developed D-3–selective compound 7-OHDPAT binds to D-3 receptors with an affinity of <1 nM and has a lower (weaker) affinity for other subtypes (about 10^2-, 10^3-, and 10^4-fold lower affinity for D-2, D-4, and D-1 receptors, respectively) (*7*). Quinpirole has approximately equal affinity for D-3 and D-4 receptors and has about 100-fold and 3000-fold lower affinity for D-2 and D-1 receptors, respectively (*7*, *8*). Apomorphine has high affinity for D-4 receptors, about 10-fold lower affinity for D-3 and D-2 receptors, and 100-fold lower affinity for D-1 receptors (*7*, *8*). Using these three dopamine agonists, we investigated the ability of D-3 receptors to modulate cocaine self-administration in the rat.

Male Wistar rats were implanted with long-term jugular catheters and trained to self-administer cocaine intravenously in daily 3-hour sessions (*9*). Once baseline rates of cocaine self-administration were established, various doses of dopamine agonists were combined with cocaine in the self-administration syringe (*9*). All of the dopamine agonists, when self-administered in combination with cocaine, reduced cocaine intake by producing an increase in the interval between injections without disrupting self-administration (Fig. 1). This is interpreted as an enhancement of cocaine's reinforcing effects because an increase in the dose of cocaine produces the same effect (*10*). The relative potencies of the various agonists to decrease cocaine self-administration were calculated from

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the graded dose-response data according to standard pharmacologic calculations (*11*); these potencies were linearly related to the inhibition constant K_i of these agents for the D-3 receptor ($r = 0.99$, $P < 0.05$) (*7*, *12*). However, no such relation was observed between the relative potencies of these compounds to reduce cocaine self-administration and their K_i values for the D-2, D-1, or D-4 receptor (*7*, *8*, *13*).

When substituted for cocaine, each agonist also maintained self-administration alone (Fig. 2) with a dose-effect curve shaped like an inverted U, which is characteristic of drug self-administration (*10*, *14*). Apomorphine self-administration has been reported in rats (*2*, *15*), and self-administration studies in primates have shown that apomorphine and quinpirole function as positive reinforcers in these species as well (*16*).

The dose-related self-administration of dopamine agonists in this study revealed a striking difference between the effects of apomorphine and the more selective D-3 agents. The effects of apomorphine on cocaine self-administration appeared to be simply additive because apomorphine (16 μg) given in combination with cocaine (0.25 mg) decreased the number of self-injections by approximately half, much

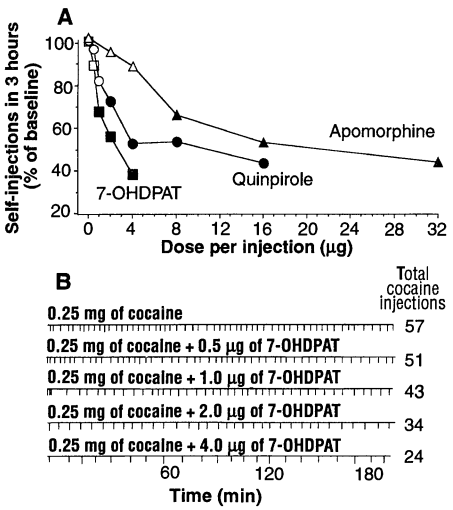

Fig. 1. (**A**) Effects of D-3 agonists on cocaine self-administration. Solid symbols indicate doses significantly different from zero by independent comparison ($P < 0.01$, Dunnett's t) (*27*). The 7-OHDPAT ($n = 7$) and quinpirole ($n = 5$) were tested in separate naïve subjects, but three animals from these studies were subsequently tested with apomorphine ($n = 5$). (**B**) The self-administration records for animal 2. Each mark indicates delivery of a cocaine infusion (0.25 mg of cocaine with 0 to 4 μg of 7-OHDPAT) after completion of five lever responses. In all cases, two priming injections were delivered immediately before the session. Animal 2 was representative, except at the highest dose of 7-OHDPAT where two animals self-administered only four injections of cocaine.

the same as a double dose of either substance (32 μg of apomorphine or 0.5 mg of cocaine) when self-administered alone (*17*). In contrast, a dose of 7-OHDPAT (4 μg) that reduced cocaine self-administration by approximately half was not reliably self-administered, and a 32-fold higher dose was required to produce patterns of self-administration that resembled cocaine self-administration patterns (Figs. 1 and 2) (*18*). A similar discrepancy between the ability to reduce cocaine self-administration and the reinforcing properties of low doses of quinpirole was observed (*19*). Thus, the data presented here suggest that D-3–selective dopamine agonists reduce cocaine self-administration at doses of these agents that are not by themselves reinforcing.

In contrast to its effects on cocaine self-administration, 7-OHDPAT (4 to 16 μg) did not alter apomorphine self-administration even at a dose fourfold higher than that which decreased cocaine self-administration by 61% (*20*). The ability of low doses of 7-OHDPAT to decrease the self-administration of cocaine but not apomorphine may be related to the different mechanisms that mediate the reinforcing properties of cocaine and apomorphine. Cocaine acts as an indirect dopamine agonist by preventing the reuptake of dopamine into dopaminergic nerve terminals, and selective destruction of those terminals abolishes cocaine self-administration (*2*). However, apomorphine directly stimulates dopamine receptors, and self-administration of apomorphine has been shown to be independent of presynaptic dopaminergic elements (*2*). Thus, D-3–selective dopamine agonists may interact presynaptically to enhance cocaine's reinforcing properties. This is consistent with observations that dopaminergic neurons express D-3 receptors (*4*) and that D-3–preferential antagonists have often been categorized as autoreceptor-selective in electrophysiological, biochemical, and behavioral studies (*21*).

Our understanding of the role of D-3 receptors in cocaine reinforcement is hindered by the absence of highly selective D-3 receptor antagonists. The most preferential D-3 antagonists available, UH232 and AJ76, have only a fourfold selectivity for D-3 over D-2 receptors (*7*). Pretreatments with low doses of UH232 (1.25 to 10 mg per kilogram of body weight, subcutaneously) produced a small but dose-dependent increase in cocaine self-administration in six animals (*22*). This relatively weak antagonism of cocaine's reinforcing properties is in agreement with findings that these compounds do not attenuate the discriminative stimulus properties of cocaine (*23*).

In comparison to other dopamine receptor subtypes, D-3 receptors are distinguished by an unusually high affinity for dopamine and a discrete localization within "limbic" dopaminergic projection areas associated with emotional, cognitive, and endocrine functions (*7*). The appreciable densities of D-3 receptors in the ventral striatum, where dopamine is implicated in cocaine reinforcement (*2*), and lack of D-3 receptors in the dorsal striatum, where dopamine modulates motor functions (*24*), suggest that D-3 agents may modulate cocaine intake without producing the motor side effects associated with other dopaminergic compounds (*25*). Comparisons of recent treatments for cocaine abuse indicate that medications that diminish cocaine use and craving without producing dependence or addictive use patterns themselves hold more promise than efforts to block the effects of cocaine (*26*). The observation that D-3–selective dopamine agonists decrease cocaine self-administration at doses that are not reliably self-administered themselves is consistent with this strategy and suggests that the D-3 receptor may be a useful target for new pharmacotherapies for cocaine abuse.

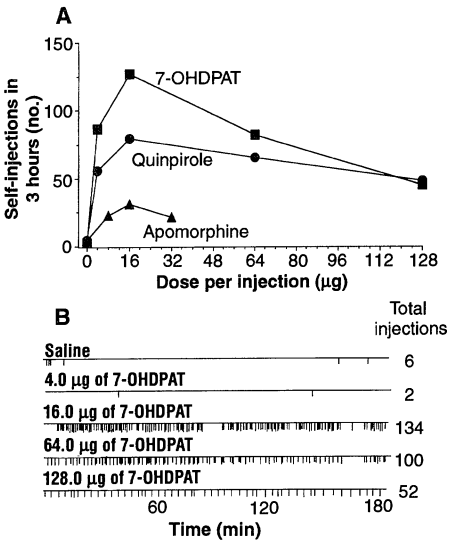

Fig. 2. (**A**) Self-administration of 7-OHDPAT, quinpirole, and apomorphine ($n = 3$ for each test; one subject was tested with both 7-OHDPAT and quinpirole, and one was tested with all three agonists). For each of the agonists, the lowest dose (4 to 8 μg) maintained robust self-administration in only one animal out of the three tested (*14*). (**B**) The self-administration records for animal 20 as in Fig. 1B, except that each mark indicates delivery of an infusion of 7-OHDPAT only (0 to 128 μg).

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10. Drug self-administration in animals exhibits a characteristic inverted U-shaped dose-response curve, and manipulations that shift this curve to the left can be interpreted as increasing the reinforcing potency of the drug. Pretreatments with dopamine agonists before the session have been shown to produce this effect by decreasing the frequency of cocaine self-injections [C. B. Hubner and G. F. Koob, *Neuropsychopharmacology* **3**, 101 (1990)]. Alternatively, this effect can be interpreted as enhancing the rate-decreasing effects of cocaine [S. Herling, D. A. Downs, J. H. Woods, *Psychopharmacology Berlin* **64**, 261 (1979)].
11. Relative potency is the ratio of the amounts of each drug needed to produce the specified effect; a higher numerical value indicates a weaker relative potency. The relative potencies of the agonists to decrease cocaine self-administration were calculated from comparisons of the dose (micromoles) response; 7-OHDPAT was assigned unit potency (1.0). The relative potencies were 3.22 for quinpirole and 8.97 for apomorphine (the correlation coefficients of the regression lines for the potency estimates were $r = 0.79$, $r = 0.85$, and $r = 0.88$, respectively) [R. J. Tallarida and R. B. Murray, *Manual of Pharmacologic Calculations with Computer Programs* (Springer-Verlag, New York, 1987), pp. 35–38].
12. The regression analysis indicated a significant correlation of $r = 0.99$ [$F(1,2) = 414.4$, $P < 0.05$].
13. The regression analyses indicated no significant positive correlation between the relative potencies of the three agonists to decrease cocaine self-administration and their K_i values for the D-2, D-1, or D-4 receptor. Indeed, the correlation coefficients for the regression lines were negative ($r = -0.31$, $r = -0.68$, and $r = -0.75$, respectively, $P > 0.1$ in each case).
14. Low doses of the agonists were not sufficiently reinforcing to produce self-administration in two of the three animals tested with each drug (4 μg of 7-OHDPAT: 2, 2, and 258 injections in 3 hours; 4 μg of quinpirole: 2, 2, and 164 injections in 3 hours; and 8 μg of apomorphine: 0, 11, and 58 injections in 3 hours), but as the dose was increased, characteristic patterns of self-administration were produced in all animals such that the dose per injection was inversely related to the total number of self-injections.
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17. Total number of self-injections in 3 hours (mean ± SEM, $n = 3$, same subjects): cocaine (0.25 mg) alone, 37.4 ± 3.5; apomorphine (16 μg) in combination with cocaine (0.25 mg), 21.2 ± 0.8; apomorphine (32 μg) alone, 21.4 ± 0.7. A double dose of cocaine (0.5 mg) has been shown to reduce the total number of cocaine self-injections by about half [G. F. Koob, F. J. Vaccarino, M. Amalric, F. E. Bloom, in *Brain Reward Systems and Abuse*, J. Engel and L. Oreland, Eds. (Raven, New York, 1987), pp. 35–50].
18. As in (*17*): cocaine (0.25 mg) alone, 46.8 ± 3.0; 7-OHDPAT (4 μg) in combination with cocaine (0.25 mg), 24.0 ± 2.4; 7-OHDPAT (128 μg) alone, 45.0 ± 6.5.
19. As in (*17*): cocaine (0.25 mg) alone, 43.4 ± 0.8; quinpirole (4 μg) in combination with cocaine (0.25 mg), 23.2 ± 1.9; and quinpirole (128 μg) alone, 48.3 ± 16.8.
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28. We thank R. Amstutz, D. Römer, E. Rissi, and P. Seiller of Sandoz Inc., Basel, Switzerland, for providing 7-OHDPAT. We also thank F. E. Bloom for comments throughout these studies and B. Everitt, L. Gold, G. Schulteis, A. Markou, and B. Baldo for comments on the manuscript. Supported by National Institute on Drug Abuse (NIDA) grant DA04398 to G.F.K. and NIDA predoctoral fellowship DA05478 to S.B.C. All animal procedures conformed to the *Guide for the Care and Use of Laboratory Animals* endorsed by the National Institutes of Health. This is publication 7732-NP from The Scripps Research Institute.

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Molecular Cloning of an Apolipoprotein B Messenger RNA Editing Protein

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Mammalian apolipoprotein B (apo B) exists in two forms, each the product of a single gene. The shorter form, apo B48, arises by posttranscriptional RNA editing whereby cytidine deamination produces a UAA termination codon. A full-length complementary DNA clone encoding an apo B messenger RNA editing protein (REPR) was isolated from rat small intestine. The 229-residue protein contains consensus phosphorylation sites and leucine zipper domains. HepG2 cell extracts acquire editing activity when mixed with REPR from oocyte extracts. REPR is essential for apo B messenger RNA editing, and the isolation and characterization of REPR may lead to the identification of other eukaryotic RNA editing proteins.

Apolipoprotein B is a critical structural component of circulating lipoproteins and a major etiologic factor in atherosclerosis susceptibility (*1*). The liver synthesizes a 550-kD form of apo B, apo B100, whereas a smaller form, apo B48, is produced from the small intestine by posttranscriptional editing of a CAA (glutamine) to a UAA (stop) codon in apo B mRNA (*2*, *3*). The truncated apo B48 protein lacks the receptor-binding domains for low-density lipoprotein present in apo B100 and is therefore catabolized by a different receptor pathway (*4*, *5*). Intestinal apo B mRNA editing is developmentally regulated in humans and other mammals (6–8) and appears to be mediated by a protein factor or factors that are sequence-, tissue-, and species-specific (*9–18*).

We developed a functional complementation assay for apo B RNA editing on the basis of our recent observation (*18*) that chicken enterocyte S100 extracts enhance in vitro editing of mammalian apo B RNA (Fig. 1) in spite of the fact that chicken apo B mRNA is not itself edited. Rat intestinal polyadenylated [poly(A)$^+$] RNA was size-fractionated by sucrose gradient ultracentrifugation (*19*) and injected into *Xenopus* oocytes. One fraction (fraction 4, Fig. 1) yielded editing activity in oocyte extracts, a function dependent on the addition of chicken enterocyte S100 extract. This functionally active mRNA fraction was used to construct a plasmid cDNA library that contained ~1 × 10^6 cDNA clones. Screening

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