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Adaptive Increase in D_3 Dopamine Receptors in the Brain Reward Circuits of Human Cocaine Fatalities

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The mesolimbic dopaminergic system plays a primary role in mediating the euphoric and rewarding effects of most abused drugs. Chronic cocaine use is associated with an increase in dopamine neurotransmission resulting from the blockade of dopamine uptake and is mediated by the activation of dopamine receptors. Recent studies have suggested that the D_3 receptor subtype plays a pivotal role in the reinforcing effects of cocaine. The D_3 receptor-preferring agonist 7-hydroxy-*N,N*-di-*n*-propyl-2-aminotetralin (7-OH-DPAT) is a reinforcer in rhesus monkeys trained to self-administer cocaine, but not in cocaine-naive monkeys. *In vitro* autoradiographic localization of [^{3}H]-(+)-7-OH-DPAT binding in the human brain demonstrated that D_3 receptors were prevalent and highly localized over the ventromedial sectors of the striatum. Pharmacological characterization of [^{3}H]-(+)-7-OH-DPAT binding to the human nucleus accumbens demonstrated a rank order of potency similar to that observed for binding to the cloned D_3 receptor expressed in transfected cell lines. Region-of-interest analysis of [^{3}H]-(+)-7-OH-DPAT binding to the D_3 receptor demonstrated a one- to threefold elevation in the number of binding sites over particular sectors of the striatum and substantia nigra in cocaine overdose victims as compared with age-matched and drug-free control subjects. The elevated number of [^{3}H]-(+)-7-OH-DPAT binding sites demonstrates that adaptive changes in the D_3 receptor in the reward circuitry of the brain are associated with chronic cocaine abuse. These results suggest that the D_3 receptor may be a useful target for drug development of anti-cocaine medications.

Key words: cocaine; human; brain; D_3 receptor; (+)-7-OH-DPAT; density

The reinforcing effects of cocaine are mediated by the potentiation of dopamine (DA) neurotransmission. Cocaine binds to the presynaptic DA transporter and inhibits the reuptake of released DA (Ritz et al., 1987; Reith et al., 1989; Kuhar et al., 1991). Increased intrasynaptic DA interacts with pre- and postsynaptic DA receptors to initiate a sequence of events that mediate the reinforcing effects of cocaine (Koob and Bloom, 1988; Kuhar et al., 1991; Pulvirenti and Koob, 1994). DAergic signaling is mediated by five receptor subtypes distinguished by their unique molecular and pharmacological properties and distinct anatomical locations. Interest has focused on the D_3 receptor because of the association of this DA receptor subtype with the mesolimbic reward circuits in brain.

The DAergic system in the nucleus accumbens is a neuroanatomical substrate for cocaine reinforcement (Koob and Bloom, 1988; Robledo et al., 1992). In the human brain, D_3 receptor mRNA and binding sites are prevalent throughout the ventral and medial sectors of the striatum (Landwehrmeyer et al., 1993b; Murray et al., 1994). The human D_3 receptor cDNA encodes a protein with 400 amino acids, which shares 46% homology overall and 78% homology within the transmembrane (TM) domains of the human D_2 receptor (Giros et al., 1990; Sokoloff et al., 1990, 1992a,c). The D_3 receptor gene is structurally complex, with the coding sequence interrupted by introns that may be spliced alternatively to generate receptor isoforms (Giros et al., 1990, 1991; Sokoloff et al., 1992a). Three variants of the human D_3 receptor have been identified to date, including the D_3(TM3-del) (Snyder et al., 1991), D_3(TM4-del) (Nagai et al., 1993), and D_{3nf} (Liu et al., 1994). Although the functional significance of less abundant and shorter mRNA species is still unclear, one possibility is that atypical regulatory processing of the mRNA in response to chronic agonist or antagonist stimulation may lead to the expression of truncated D_3 receptor proteins (Liu et al., 1994).

Although the precise contribution of each of the DA receptor subtypes to the behavioral effects of cocaine is not understood fully, recent studies suggest that there are a number of promising DAergic subtype-selective agents that deserve further evaluation as potential therapies for cocaine abuse (Robledo et al., 1992; Pulvirenti and Koob, 1994; Roberts and Ranaldi, 1995). Studies by Caine and Koob (1993, 1995) have suggested that the D_3 receptor may be a primary mediator of the reinforcing effects of cocaine. D_3 receptor-preferring agonists, although not self-administered by drug-naive monkeys, are reinforcing in monkeys that have been trained to self-administer cocaine (Nader and Mach, 1996). D_1 (SKF 81297) and putative D_3 (7-OH DPAT) agonists exert qualitatively different aspects of the reinforcing stimulus produced by cocaine (Self et al., 1996), and the D_3 antagonist (DS 121) attenuates the motivation to self-administer cocaine in rats (Roberts and Ranaldi, 1995). Chronic exposure to cocaine leads to regulatory adaptations in the regional complement of specific DA receptor subtypes, which in turn may affect the expression of the reinforcing effects of cocaine. In the present study, we used [^{3}H]-(+)-7-OH-DPAT *in vitro* for ligand binding and autoradiographic mapping to investigate the regulatory effects of cocaine on the D_3 receptor in human brain.

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Table 1. Pharmacological profile of [^{3}H]-(+)-7-OH-DPAT binding to the D_3 receptor

	Human nucleus accumbens		Cloned D_3 receptor
Competitor	n_H	K_I, (nM)	K_I, (nM)
Dopamine agonists			
(+)-7-OH DPAT	0.67	3.1 ± 1.1	0.8 ± 0.1[a]
PD 128907	0.70	6.5 ± 0.2	
(−) Quinpirole	0.68	12.8 ± 0.9	3.7 ± 0.2[a]
			5.1 ± 0.3[b]
			29.4 ± 4.0[c]
Dopamine	0.84	15.6 ± 2.3	43.9 ± 4.4[a]
			25.0 ± 3.0[b]
Dopamine antagonists			
(−) Eticlopride	0.95	0.1 ± 0.03	0.2 ± 0.02[d]
Spiperone	0.72	0.3 ± 0.07	0.3 ± 0.04[d]
			0.6 ± 0.05[b]
Pimozide	0.84	2.5 ± 1.5	3.7 ± 0.5[b]
Raclopride	0.78	2.9 ± 0.8	3.5 ± 0.3[b]
(+) Butaclamol	0.81	3.7 ± 0.6	11.2 ± 0.8[d]
			4.1 ± 1.2[c]
Domperidone	0.77	8.5 ± 0.4	9.5 ± 0.5[b]
(+) AJ76	0.77	94.4 ± 0.5	
			389.0
Clozapine	0.85	304.6 ± 8.5	± 31.0[a]
			480.0 ± 47.4[c]
			180.0
			± 17.0[b]
(+) Pentazocine	1.00	1144.7 ± 341.3	
(−) Pentazocine	0.82	3646.4 ± 443.5	

The potency values for inhibition of [^{3}H]-(+)-7-OH DPAT (1 nM) binding to nucleus accumbens membranes are shown.

[a] D_3-HEK293 cells (Burris et al., 1994).

[b] D_3-CHO cells (Sokoloff et al., 1990, 1992d).

[c] D_3MN9D cells (MacKenzie et al., 1994).

[d] D_3-CCL1.3 (MacKenzie et al., 1994).

MATERIALS AND METHODS

Materials. [^{3}H]-(+)-7-OH DPAT was purchased from Amersham (Arlington Heights, IL). All unlabeled drugs were obtained from Research Biochemicals (Natick, MA), with the exception of the pentazocine isomers that were supplied by the National Institute on Drug Abuse Drug Supply (Rockville, MD), and (+) AJ76, which was generously supplied by Upjohn (Kalamazoo, MI). Tritium standards and Hyperfilm for autoradiographic studies were purchased from Amersham.

Neuropathological tissue specimens. Postmortem neuropathological specimens were obtained at autopsy from age-matched and drug-free control subjects (n = 9; mean age = 30.0 ± 2.8 years; mean autolysis = 15.0 ± 1.6 hr), cocaine overdose (CO) victims (n = 6; mean age = 32.2 ± 2.2 years; mean autolysis = 18.5 ± 2.4 hr), and excited delirium (ED) victims (n = 6; mean age = 32.3 ± 2.3 years; mean autolysis = 11.2 ± 1.1 hr). Medicolegal investigations of the deaths were conducted by forensic pathologists who evaluated the scene environment and circumstances of death, performed autopsies on the victims, and determined the cause and manner of death (Mittelman and Wetli, 1984). The circumstances of death and toxicology data were reviewed carefully before a death was classified as a CO (Escobedo et al., 1991; Wetli et al., 1996). In a similar manner, controls were selected from those whose deaths were not caused by cocaine, with no cocaine or metabolites detected in toxicology screens of blood or brain tissues. All cases were evaluated for common drugs of abuse and alcohol, and positive urine screens were confirmed by quantitative analysis of blood. Alcohol was detected in two of the control subjects (0.01–0.05%) and in one of the CO victims (0.05%). The cocaine toxicity cases selected for the present study had evidence of a number of different variables of chronic cocaine use, determined on the basis of review of the previous arrest records and treatment admissions as well as on pathological signs (i.e., perforation of the nasal septum). Blood cocaine was quantified using gas–liquid chromatography with a nitrogen detector. Frozen brain regions were sampled for quantitation of cocaine and benzoylecgonine using gas chromatography/mass spectroscopy techniques (Hernandez et al., 1994). Neuropathological analysis was carried out to verify the absence of any gross or histopathological abnormalities.

Ligand binding assays. Putative D_3 receptors were labeled using the procedure described by Burris et al. (1994), with some minor modifications. Briefly, tissue punches from the nucleus accumbens were weighed, homogenized in (1:20, w/v) ice-cold 10 mM Tris-HCl buffer, pH 7.4, 5 mM EDTA, and centrifuged for 20 min at 32,000 × g. Membranes were washed once in Tris-HCl buffer, pH 7.7, 1.0 mM EDTA, and resuspended in assay buffer that contained 50 mM Tris, pH 7.7, 2 mM $MgCl_2$, and 50 mM NaCl. The guanine nucleotide GTP (300 μM) was included in the assay tubes to enhance the selectivity of [^{3}H]-(+)-7-OH-DPAT binding to D_3 receptors over D_2 receptors. For saturation binding, increasing concentrations of [^{3}H]-(+)-7-OH-DPAT were incubated with nucleus accumbens membranes (5 mg tissue original wet weight) in the presence and absence of 10 μM (+)-butaclamol for 2 hr at 25°C. Competition binding assays were conducted with various concentrations of competitor incubated in the presence of 1 nM [^{3}H]-(+)-7-OH-DPAT for 2 hr at 25°C. The binding reaction was terminated by dilution with 4 ml of ice-cold 50 mM Tris-HCl, pH 7.7, and bound radioligand was separated from free radioligand by vacuum filtration through 934AH filters presoaked in 0.1% polyethyleneimine. Filters were washed three times with 4 ml of ice-cold buffer and counted by a Beckman Scintillation counter at 50% efficiency.

In vitro *autoradiography.* Half-hemisphere coronal sections of the human brain were cut on a Hacker/Bright sledge microtome cryostat at 50 μm, thaw-mounted on gelatin-coated slides, and dried under reduced pressure at 4°C. Adjacent sections were stained with Nissl substance and acetylcholinesterase for cytoarchitecture. For D_3 receptor autoradiography, slide-mounted tissue sections were incubated with 1 nM [^{3}H]-(+)-7-OH-DPAT in the presence of 300 μM GTP for 2 hr at 25°C. Nonspecific binding was determined in the presence of 10 μM (+)-butaclamol. At the end of the incubation, tissue sections were washed in two changes of ice-cold assay buffer followed by a quick rinse in ice-cold distilled water to dissociate nonspecifically bound ligand. Tissue sections were dried under a stream of cool air and apposed with tritium standards to Hyperfilm for 7–8 weeks at 4°C.

Data analysis. For analysis of binding data, equilibrium binding constants were determined from the saturation binding data using the iterative, nonlinear curve-fitting program EBDA/LIGAND, (Biosoft, Elsevier). The best fit to a one- or two-site model was based on the partial F-test. The rank order of potency for [^{3}H]-(+)-7-OH-DPAT binding was determined by competition binding analysis; IC_{50} and K_i values were determined using DRUG (EBDA) and LIGAND, respectively. Differences in D_3 receptor densities between control subject and experimental groups were analyzed for statistical significance by the Student's t test.

For analysis of D_3 receptor autoradiography, films were scanned using a Howtek Scanmaster 3 at 400 dots per inch using a transparency illuminator. The resulting tagged image file format for RGB color files were converted to pseudocolor format in specific activity units using the IMAGE (version 1.44; National Institutes of Health Shareware) and BRAIN (version 1.6; Drexel University) programs. After background subtraction, two-dimensional pseudocolor maps were created to allow radioactivity levels (in fmol/mg) to be superimposed on the sections (Kuhar et al., 1986).

RESULTS

Characteristics of fatal CO victims

CO deaths are defined as deaths that were investigated and on the basis of medical judgment were attributed to the toxic effects of cocaine alone or in combination with alcohol. Cocaine fatalities were identified and classified as part of an ongoing case-control study of the toxicology reports, scene descriptions, supplemental background information, and autopsy findings (Escobedo et al., 1991). On the basis of this analysis, CO cases demonstrating evidence of significant underlying cardiac pathology, cerebrovascular disorders, or polydrug abuse were eliminated from the study. CO deaths presenting with preterminal ED have been included in this study as a comparison group. This syndrome is composed of

four components that appear in sequence: hyperthermia, delirium with agitation, respiratory arrest, and death (Wetli and Fishbain, 1985; Wetli et al., 1996).

The concentration of cocaine and its principal metabolite benzoylecgonine (BE) were measured in blood and brain samples obtained at autopsy. All cocaine fatalities had quantifiable levels of cocaine in blood and brain. The average (mean ± SEM) blood levels of cocaine and BE were 9.2 ± 3.8 and 8.1 ± 2.2 mg/l in the CO victims. The ED victims exhibited 10-fold lower levels of cocaine (0.6 ± 0.2 mg/l) and 4-fold lower levels of BE (1.9 ± 0.5 mg/l) in blood. The levels of cocaine and BE were measured also in brain tissue specimens from occipital cortex (Brodman's areas 17 and 18). The mean cocaine and BE levels in the CO group were 12.8 ± 4.0 and 3.8 ± 1.3 mg/kg tissue, respectively. Similar to the observations in blood samples, the ED victims exhibited ~10-fold lower levels of cocaine (1.2 ± 0.3 mg/kg) and twofold lower levels of BE (1.9 ± 0.5 mg/kg) in brain. Elevated body temperatures were recorded for five of the fatal ED victims (range, 101.7–110.0°C; mean ± SEM, 104.4 ± 1.5°C).

Visualization of D_3 receptor distribution in human brain

Pharmacological studies with [^{3}H]-(+)-7-OH-DPAT demonstrate that it has a 100-fold higher affinity for binding to the cloned D_3 receptor as compared with the cloned D_2 receptor expressed in transfected cell lines. Binding studies conducted in brain in regions enriched in the native D_2 and D_3 receptors have indicated that [^{3}H]-(+)-7-OH-DPAT demonstrates selectivity for the D_3 subtype when the receptors are dissociated from their respective G-proteins (Large and Stubbs, 1994). This selectivity profile ($D_3 > D_2$) is not seen in the absence of guanine nucleotides, because the high-affinity G-protein-coupled state of the D_2 receptor is left-shifted and overlaps in binding affinity with that of the D_3 receptor. Because guanine nucleotides have a minimal effect on agonist binding to the D_3 receptor (twofold right-shift), but markedly decrease agonist binding to the D_2 receptor (100-fold right-shift), it is possible to achieve selective labeling of the D_3 receptor in the presence of GTP (Burris et al., 1994). Under these assay conditions, [^{3}H]-(+)-7-OH-DPAT labels a single population of binding sites in the human nucleus accumbens with an affinity value (K_D) of 1.3 ± 0.3 nM (mean ± SEM) and a density (B_{max}) of 2.8 ± 0.3 pmol/gm tissue original wet weight. The pharmacological profile for binding of [^{3}H]-(+)-7-OH-DPAT to the D_3 receptor in human nucleus accumbens is shown in Table 1. The putative D_3 agonists (+)-7-OH-DPAT and PD128907 demonstrated the highest potencies for inhibition of [^{3}H]-(+)-7-OH-DPAT binding. Quinpirole and DA exhibited twofold lower potency as compared with (+)-7-OH-DPAT and PD128907. The D_2 receptor antagonists (−)-eticlopride and spiperone were the most potent inhibitors of [^{3}H]-(+)-7-OH-DPAT binding. Pimozide, raclopride, (+)-butaclamol, and domperidone had lower potencies, whereas (+)-AJ 76 and clozapine were the least potent of the dopamine antagonists. The isomers of the σ-active drug pentazocine inhibited binding of [^{3}H]-(+)-7-OH-DPAT with micromolar potencies. The overall rank order of inhibition of [^{3}H]-(+)-7-OH-DPAT binding observed in human nucleus accumbens correlated significantly with the K_i values reported previously for the cloned D_3 receptor ($r = 0.98$; $p < 0.001$; Table 1).

The regional distribution of the D_3 receptor was mapped in half-hemisphere coronal sections of the human brain. *In vitro* autoradiographic localization of [^{3}H]-(+)-7-OH-DPAT binding demonstrated high densities of D_3 receptors in the nucleus accumbens and the ventromedial sectors of the striatum (Fig. 1). Moderate densities were observed in the dorsal sectors of the anterior caudate and putamen. Low levels of labeling were apparent also in the hypothalamus, reticular thalamus, and substantia nigra. Low levels of labeling were observed in the entorhinal and cingulate gyri and over the frontal and parietal lobes.

Regulation of the D_3 receptor by cocaine

Quantitative *in vitro* autoradiography was used to map and quantify D_3 receptor densities in human CO victims. Binding of [^{3}H]-(+)-7-OH-DPAT was elevated approximately twofold in the ventromedial sectors of the anterior caudate and putamen and in the nucleus accumbens of the CO victims as compared with drug-free and age-matched control subjects (Fig. 2). The intensity of [^{3}H]-(+)-7-OH-DPAT labeling was increased also in the lateral and medial divisions of the substantia nigra in the CO victims. Binding of [^{3}H]-(+)-7-OH-DPAT was not elevated significantly in the anterior ventral striatum of the ED subgroup; however, preliminary studies indicate a two- to threefold elevation in receptor densities over the cell body fields in the medial division of the substantia nigra. Quantitative densitometric measurements of [^{3}H]-(+)-7-OH-DPAT binding revealed significant elevations in the densities of D_3 receptors throughout the mesolimbic sectors of the striatum in the CO victims as compared with the drug-free and age-matched control subjects (Fig. 3; Student's t test; $p < 0.05$). These findings were confirmed further by saturation analysis of [^{3}H]-(+)-7-OH-DPAT binding in membrane homogenates from the nucleus accumbens. The affinity for [^{3}H]-(+)-7-OH-DPAT binding was not different in the CO victims (K_D = 1.5 ± 0.2 nM; $n = 6$) or the ED victims (1.7 ± 0.4 nM; $n = 6$) as compared with drug-free control subjects (K_D = 1.3 ± 0.3 nM; $n = 5$). Figure 4 illustrates the lack of a change in the affinity for [^{3}H]-(+)-7-OH-DPAT binding to the D_3 receptor in the nucleus accumbens of a representative CO victim as compared with a representative drug-free and age-matched control subject. The saturation binding density for the CO victims (4.6 ± 0.4 pmol/gm) when compared with the drug-free control subjects (2.8 ± 0.3 pmol/gm) was significantly elevated (Student's t test; $p < 0.001$). The density for [^{3}H]-(+)-7-OH-DPAT binding to the nucleus accumbens in the ED victims was not significantly different (4.0 ± 1.0 pmol/gm) from control values.

DISCUSSION

We have investigated the effect of cocaine exposure on the affinity and number of D_3 receptors in human brain. The regulatory profile shown here provides additional support for a role of the D_3 receptor in the modulation of addictive behaviors, including cocaine dependence. In human CO victims, D_3 receptor number was increased as compared with drug-free and age-matched control subjects in the nucleus accumbens, mesolimbic sectors of the caudate and putamen, and substantia nigra. These findings suggest that cocaine use may lead to an adaptive elevation in D_3 receptor density in response to elevated synaptic DA levels.

Pharmacological signature and anatomical locations of the D_3 receptor in human brain

The specificity of [^{3}H]-(+)-7-OH-DPAT labeling in human brain was confirmed by saturation analysis and competition binding assays. Saturation analysis revealed a single high-affinity binding site with a K_D value comparable to that observed for the cloned D_3 receptor (Levesque et al., 1992; Chio et al., 1994; MacKenzie et al., 1994; Pilon et al., 1994). Competition binding assays demonstrated a rank order of potency [(−)-eticlopride ≥ spiperone > pimozide = raclopride = (+)-7-OH-DPAT = (+)-butaclamol ≥

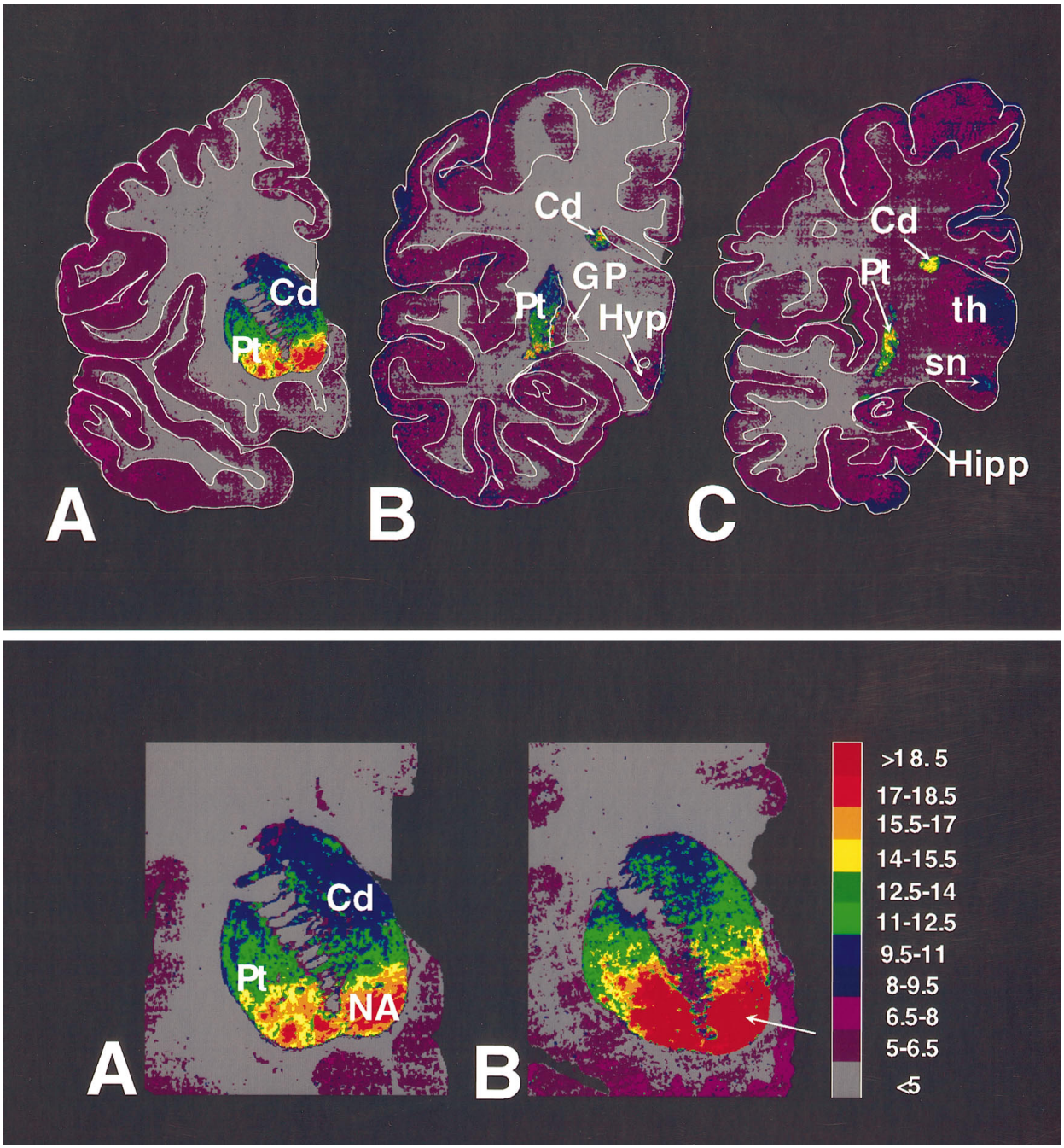

Figure 1. **Top.** Autoradiographic localization of [^{3}H]-(+)-7-OH-DPAT binding in representative half-hemisphere coronal sections of human brain. Computer-generated color coding of the autoradiograms from a series of half-hemisphere coronal sections of the human brain at three different anterior to posterior levels through the striatum is shown. The pseudocolor codes represent a rainbow scale (*red* = high densities; *yellow* to *green* = intermediate densities; *blue* to *purple* = low densities). High densities of the D_3 receptor were observed in the ventral sectors of the striatum, with the most prevalent labeling in the nucleus accumbens. Moderate labeling was seen in the substantia nigra. *Cd*, Caudate; *GP*, globus pallidus; *Hipp*, hippocampus; *Hyp*, hypothalamus; *Pt*, putamen; *sn*, substantia nigra; *th*, thalamus.

Figure 2. **Bottom.** Pseudocolor density maps of [^{3}H]-(+)-7-OH-DPAT binding to the D_3 receptor in the anterior striatum of (*A*) a representative drug-free control subject and (*B*) a representative CO victim. Note the significant increase in the density of the D_3 receptors throughout the mesolimbic sectors of the striatum. The *color bar* at the *right* depicts the density of radioligand binding sites in fmol/mg tissue equivalence units.

PD 128907 ≥ domperidone ≥ (−)-quinpirole = dopamine > (+)-AJ 76 > clozapine] similar to the cloned D_3 receptor (Sokoloff et al., 1990, 1992c,d; Burris et al., 1994; Mackenzie et al., 1994). Previous studies have suggested that 7-OH-DPAT may bind also to σ receptors (Wallace and Booze, 1995); however, the σ isomers (+)- and (−)-pentazocine demonstrated low micromolar potency for inhibition of [^{3}H]-(+)-7-OH-DPAT binding. Taken together, these studies confirm that [^{3}H]-(+)-7-OH-DPAT binding in human brain demonstrates a pharmacological signature characteristic of the D_3 receptor.

DAergic competitors exhibited Hill coefficients (n_H) < 1, suggesting negative cooperativity, the recognition of multiple affinity

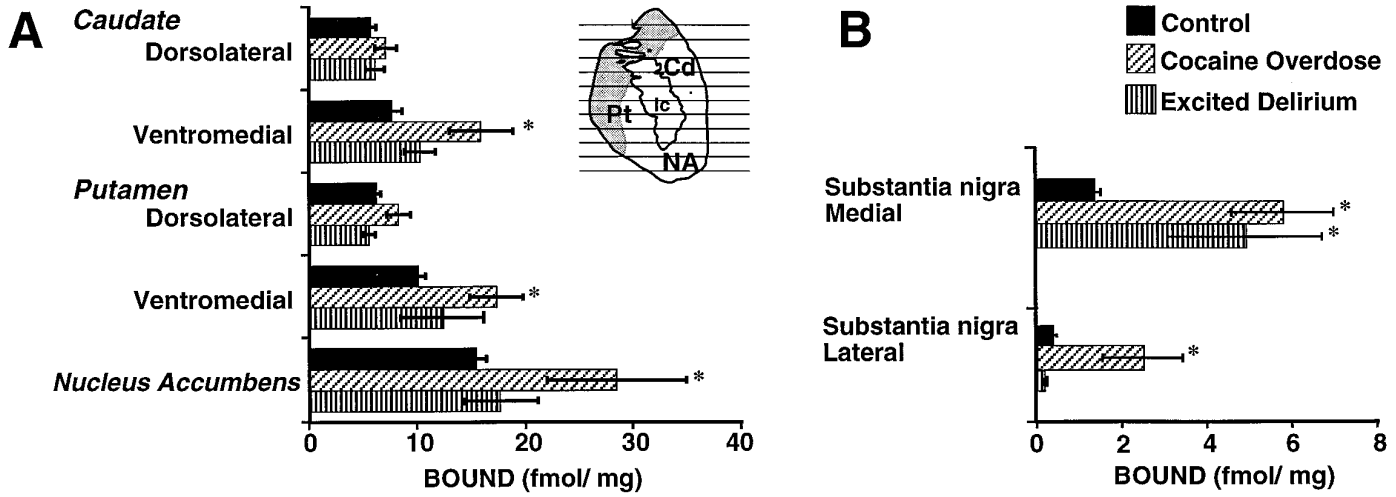

Figure 3. Summary of the region-of-interest densitometric measurements of [^{3}H]-(+)-7-OH-DPAT binding in the dopaminergic (*A*) terminal regions and (*B*) cell body fields from *Control* subjects ($n = 9$), *Cocaine Overdose* deaths ($n = 6$), and *Excited Delirium* victims ($n = 6$). The density of the D_3 receptor was determined in the substantia nigra and throughout the striatum using [^{3}H]-(+)-7-OH-DPAT. The quantitative densitometric measurements demonstrate elevated D_3 receptor densities in the ventral sectors of the striatum, including the nucleus accumbens, and in the lateral and medial sectors of the substantia nigra of the CO deaths. *Black bars* represent values for drug-free and age-matched controls; *stripped bars*, CO deaths; *stippled bars*, ED subgroup. Significant differences from control values, $^*p < 0.05$. *Cd*, Caudate; *ic*, internal capsule; *Pt*, putamen; *NA*, nucleus accumbens.

states, or distinct receptor subtypes. Both agonists and antagonists had low n_H values, and GTP was present in all assays, indicating that the binding was not to high- and low-affinity "states" of a single receptor subtype; however, 7-OH DPAT and its tetralin derivative 7-OH-PIPAT also bind to D_2 and 5-HT_{1A} receptors (Burris et al., 1994). The low n_H values may represent a minor labeling component attributable to the recognition by the radioligand of the uncoupled states of the D_2 and 5-HT_{1A} receptors. Neither of these explanations are likely, because the relative occupancies by the radioligand would be negligible for these G-protein-coupled receptors stabilized in their low-affinity conformations by sodium ion and guanine nucleotides. Alternatively, the low n_H values may represent binding to different D_3 receptor isoforms. The binding affinity of DAergic ligands may vary among the D_3 receptor isoforms. D_3(TM3-del) and D_3(TM4-del), which encode truncated D_3 receptors, do not retain sufficient tertiary structure to bind DAergic ligands (Snyder et al., 1991; Nagai et al., 1993). The D_{3nf} receptor, however, which has a frameshift deletion in the coding region of the i3 loop, may bind DAergic ligands. A purported D_3 receptor isoform, equivalent to the rat and mouse D_{3L} receptor, may exist also in human brain (Park et al., 1995). Heterogeneity in the regulatory processing of the D_3 core protein may lead to alterations in the recognition domain for the radioligand, providing an alternative explanation for the low n_H values.

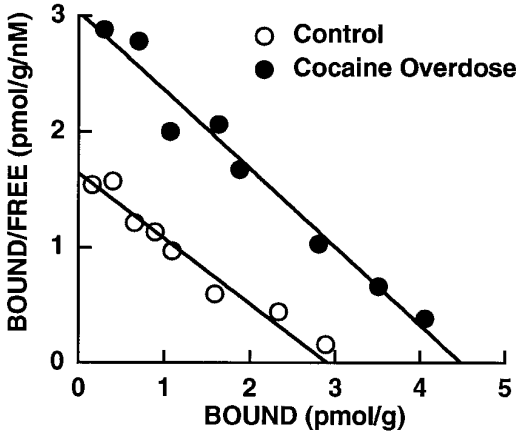

Figure 4. Rosenthal plots of [^{3}H]-(+)-7-OH-DPAT binding to nucleus accumbens in a representative control subject and a CO victim. This figure illustrates that there was no change in the affinity for [^{3}H]-(+)-7-OH-DPAT binding to the D_3 receptor, but an increase in the density of sites in the CO victim as compared with a representative age-matched and drug-free control subject.

Autoradiographic localization of [^{3}H]-(+)-7-OH-DPAT binding demonstrated that D_3 receptors were prevalent over the limbic sectors of the human striatum. The present findings confirm and extend previous studies that have demonstrated enriched densities of D_3 receptors in human and rat ventral striatum using the D_2/D_3 sensitive radioligands [^{125}I]iodosulpiride, [^{3}H]CV 205 502, and [^{125}I]epidepride (Landwehrmeyer et al., 1993a,b; Parsons et al., 1993; Hillefors-Berglund and Von Euler, 1994; Murray et al., 1994; Booze and Wallace, 1995). Overall, D_3 mRNA expression closely correlates with the localization of D_3 binding sites in human brain (Landwehrmeyer et al., 1993b). The unique anatomical localization of the D_3 receptor shown here in human brain is in agreement with a previous study (Murray et al., 1994) and provides additional support for a role for the human D_3 receptor in substance abuse.

Adaptive increase in D_3 receptor density by cocaine

Quantitative *in vitro* autoradiography demonstrated a marked elevation in D_3 receptor number in CO victims as compared with drug-free and age-matched control subjects. Although a marked elevation in D_3 receptor density was observed in all CO victims, the density was not increased reliably in every subject included in the ED subgroup. The reason for heterogeneity within this subgroup of cocaine fatalities is not fully understood, although it may be related to previous history and pattern of cocaine use. Recent and repeated use of cocaine may be necessary to elevate D_3 receptor density. Alternatively, differences in the molecular processing of D_3 receptors attributable to defects in alternatively spliced transcripts might explain the lack of an increase in the D_3 binding sites in the certain ED victims. It is interesting to note that

a different mRNA species has been found in the cortices of chronic schizophrenic patients (Schmauss et al., 1993), suggesting the possibility that similar alterations in D_3 receptor expression may be involved in the psychopathology of the cocaine delirium syndrome.

Because cocaine does not interact with the D_3 receptor, the changes in D_3 receptor number must be a secondary response to the interaction of cocaine with the DA transporter. Chronic high DA levels that result from the binge use of cocaine may lead to an adaptive increase in D_3 receptor number. Chronic treatment of C6 glioma cells transfected with the D_3 receptor cDNA with DA results in an elevation in D_3 receptor number (Cox et al., 1995). These findings suggest that the adaptive increase in D_3 receptor density observed over the mesolimbic sectors of the striatum in cocaine fatalities may be regulated by synaptic levels of DA. Recent studies by Meador-Woodruff and colleagues (1995) demonstrated no change in D_3 mRNA in human cocaine abusers. These findings suggest that D_3 mRNA and binding sites may be differentially regulated by cocaine exposure. Chronic treatment of C6 glioma cells transfected with D_3 cDNA with DA agonists demonstrated no change in D_3 mRNA abundance, although the receptor number was increased (Cox et al., 1995). The elevation in D_3 receptor density was blocked by treatment with cycloheximide in this study. These observations suggest that the adaptive increase in the D_3 receptor density observed in the present study reflects an increase in receptor protein synthesis. Alternatively, elevated [^{3}H]-(+)-7-OH DPAT binding may reflect a selective increase in one of the D_3 receptor isoforms. The abundance of different mRNA splice variants may not be discerned by *in situ* hybridization. D_3-specific probes may hybridize to all of the alternative splice variants, including the truncated D_3 receptors (Fishburn et al., 1993). Because DAergic ligands may or may not bind to the proteins generated from the truncated splice variants, a dissociation between mRNA levels and binding sites may be observed. In keeping with this suggestion, the abundance of D_{2s} receptor isoform is altered after interruption of DA transmission (Martres et al., 1992). An elevation in D_2 receptor density, but not D_2 mRNA levels, was observed after chronic treatment with haloperidol. Quantitation of the D_2 receptor splice variants by PCR methods revealed an increase in D_{2s} mRNA. Additional studies are needed to determine whether this regulatory pattern for the D_3 receptor occurs in the human brain.

The role of the D_2 and D_3 receptors in cocaine dependence

The advent of subtype-selective ligands for the members of the D_1 and D_2 receptor families has made it possible to begin to discern the specific role of the DA receptor subtypes in cocaine dependence (Roberts and Ranaldi, 1995; Self et al., 1996). The elevation in D_3 receptor densities observed in the present study contrasts with our previous observations in human brain postmortem, which showed no change in D_2 receptor densities measured with [^{3}H]raclopride in CO victims (Staley et al., 1995). The effects of cocaine exposure on the D_3 receptor has not been studied in animals. Regulatory alterations in D_2-like binding sites after chronic cocaine treatment have been shown using radioligands that did not discriminate between the D_2 and D_3 receptor subtypes. Administration of cocaine in a binge-like regimen showed a transient increase in the binding of [^{3}H]raclopride in the olfactory tubercle, nucleus accumbens, and caudate–putamen (Unterwald et al., 1994). Elevations in radiolabeled spiperone binding was observed in the nucleus accumbens, olfactory tubercle, and substantia nigra after chronic administration of cocaine (Goeders and Kuhar, 1987; Kleven et al., 1990; Peris et al., 1990; Ziegler et al., 1991). The lack of selectivity of the radioligands and the observed elevations in regions rich in D_3 receptors, suggests that it is the D_3 receptor and not the D_2 receptor that is upregulated after chronic cocaine exposure.

Implications for cocaine dependence

The neuroadaptations of the D_3 receptor that result from repeated activation of DA transmission attributable to chronic binge use of cocaine may contribute to the development of cocaine dependence. Putative D_3 receptor agonists decrease cocaine self-administration in rats (Caine and Koob, 1993, 1995) and monkeys (Nader and Mach, 1996). D_3 receptor agonists substitute for the discriminative stimulus effects of cocaine and produce place preference, indicating that the D_3 receptor may mediate some of the subjective effects of cocaine (Mallet and Beninger, 1994; Acri et al., 1995). Furthermore, 7-OH-DPAT functions as a reinforcer in monkeys trained to self-administer cocaine, but not in cocaine-naive monkeys (Nader and Mach, 1996). The behavioral studies are confounded by the lack of purported selectivity of 7–OH-DPAT for D_3 (or D_2-like) receptors *in vivo* (Large and Stubbs, 1994; Self et al., 1996). Because selective labeling of the D_3 receptor can be achieved *in vitro*, it may be suggested that our demonstration of an adaptive increase in human D_3 receptor densities by cocaine exposure may link this DA receptor subtype to the reinforcing effects of cocaine and the development of cocaine dependence.

The search for pharmacotherapies for cocaine addiction has focused primarily on drugs that target DAergic synapses. Both DA agonists and antagonists have failed to demonstrate therapeutic efficacy in cocaine dependence (Roberts and Ranaldi, 1995). Although DA agonists reduce craving, they may be reinforcing, and although DA antagonists attenuate reinforcement, compliance is hindered by dysphoria and extrapyramidal side effects. The close association of the D_3 receptor with mesolimbic DAergic circuits suggests that partial blockade of the D_3 receptor may selectively decrease the rewarding effects of cocaine without contributing to the dysphoria associated with cocaine withdrawal.

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