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Loss of Dopamine D_2 Receptors in Alzheimer's Disease with Parkinsonism But Not Parkinson's or Alzheimer's Disease

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A significant proportion of patients with Alzheimer's disease (AD) exhibit extrapyramidal features that are referred to as parkinsonism (AD/Park) to distinguish the clinical and pathological features that differ from Parkinson's disease (PD). Previous results from this laboratory have shown that, although the presynaptic components of the dopamine (DA) system are markedly affected in AD/Park, the pathology is not similar to PD (Murray et al. 1995; Joyce et al. 1997). In the present study, we determined whether the parkinsonian symptoms in AD/Park might also reflect changes in numbers of postsynaptic DA receptors. We analyzed the binding of [^{125}I]epidepride biding to DA D_2/D_3 receptors and [^{3}H]SCH 23390 to D_1 receptors by autoradiography in the striatum of six patients with PD, nine patients with AD, seven patients with AD/Park, and 14 neurologically intact control subjects. D_2 receptors were reduced in the caudate and putamen of the AD/Park group (by 42 and 27% of controls, respectively) but not reduced in AD or PD. D_1 receptors were elevated by 36% in the putamen of the PD group. Dopamine receptor changes are, therefore, not similar in PD, AD, and AD/Park. The elevation in D_1 receptors in PD may contribute to the unwanted side effects of L-dopa treatment. The loss of D_2 receptors in AD/Park, not observed in AD lacking overt parkinsonian symptomatology, may contribute to the presence of parkinsonian features and lack of responsiveness to L-dopa. ***[Neuropsychopharmacology 19:472–480, 1998]*** *Published by Elsevier Science Inc.*

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The signs and symptoms of Parkinson's Disease (PD) are thought to be attributed to degeneration of the nigrostriatal dopamine (DA) system and resulting depletion of DA from the striatal complex (Bernheimer et al. 1973; Hornykiewicz and Kish 1986). There is a greater than 85% loss of DA and DA uptake sites associated with DA fibers in PD striatum, which is more pronounced in the caudal than the rostral areas of the striatum (Kish et al. 1988; Murray et al. 1995). Parkinsonian features, particularly rigidity and bradykinesia, and infrequently tremor, are present in at least 30% of patients with Alzheimer's disease (AD) (Merello et al. 1994; Mölsä et al. 1984; Tyrrell et al. 1990; Ditter and Mirra 1987). Some studies have identified pathologic alterations similar to those observed in PD, but they occur in only a small percentage of the AD cases with extrapyramidal features (Ditter and Mirra; Leverenz and Sumi 1986; Liu et al. 1997; Murray et al. 1995; Mölsä et al. 1987). Other evidence suggests a more complex disturbance in the biochemistry of the nigrostriatal DA system in these patients. Positron emission tomography (PET) studies with ^{18}F-fluorodopa uptake show no evi-

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dence for marked loss of DA terminals in AD cases with parkinsonian signs (Tyrrell et al.). Our studies of the neurons of origin of the nigrostriatal DA system in AD cases with parkinsonian features (AD/Park) indicate that they are not extensively depleted and are capable of synthesizing tyrosine hydroxylase, but are markedly affected in the synthesis of DA transporter (DAT) normally expressed at terminal sites in the striatum (Murray et al. 1995; Joyce et al. 1997). Thus, presynaptic components of the nigrostriatal DA system in patients with AD/Park are severely affected, but the underlying pathology is distinct from that of PD. It is reasonable to hypothesize that these alterations in the presynaptic DA system can contribute to the extrapyramidal symptoms of AD/Park, because it has been shown that mice lacking the DAT exhibit a deficiency in DA release (Giros et al. 1996). Nonetheless, other components of the DA system may also contribute to the parkinsonism in AD/Park.

The actions of DA are mediated by its interaction with two basic types of G protein-coupled receptors, D_1 and D_2, which stimulate and inhibit, respectively, the enzyme adenyl cyclase (Sibley et al. 1993). Treatment of parkinsonism by chronic administration of levodopa (L-dopa)requires appropriate expression of these receptors in the striatum. A recent study using single photon emission computed tomography (SPET) identified a reduced number of D_2 receptors in the striatum of AD (Pizzolato et al. 1996). The authors hypothesized that a loss of D_2 receptors could contribute to the parkinsonian features that were evident in a number of the cases. Moreover, the decline in the number of postsynaptic striatal D_2 receptors could be one of the characteristic pathologic changes of AD, which would distinguish it from PD, in which the number of D_2 receptors is not reduced (Joyce 1993). Several studies of postmortem brains have also found losses of D_2 and/or D_1 receptors in the striatum of AD cases (Cortés et al. 1988; Cross et al. 1984; Reisine et al. 1978; Rinne et al. 1986). However, those studies made no attempt to distinguish between AD with and without parkinsonian features and the relationship to DA receptor changes in the striatum. To that end we characterized the patterns of DA receptor changes in cases of AD, PD, and AD/Park as compared to neurologically intact controls. We used [^{3}H]SCH 23390 to label D_1 receptors and [^{125}I]epidepride to label D2-like receptors with receptor autoradiography in order to be able to characterize the patterns of DA receptor changes in these groups as compared to neurologically intact controls.

METHODS

Human Tissue Acquisition and Preparation

Brain tissue was obtained at autopsy from six patients with PD confirmed by histopathology, nine patients with confirmed AD, seven patients with AD/Park, and 14 neurologically intact control subjects. The subjects were matched as closely as possible with respect to age, sex, and times of postmortem interval (Table 1). Brain tissues were obtained from the Hospital of the University of Pennsylvania (J. Trojanowski, Director of Medical Pathology) and the UCIMC Organ and Tissue Bank (graciously provided by Dr. C. Cottman, University of California, Irvine, CA). The mean (±SEM) ages and mean intervals between death and freezing of the brain were not significantly different ($p > .05$, ANOVA). Both AD and PD were diagnosed clinically and pathologically according to the methods of Arai et al. (1992). Briefly, PD was diagnosed by standard clinical criteria and their response to antiparkinsonian drugs (e.g., L-dopa). PD was confirmed at autopsy by standard pathologic criteria (e.g., presence of Lewy bodies and loss of pigmented neurons in SN). The diagnosis of AD was established according to the pathologic criteria recommended by the National Institute of Aging (Khachaturian 1985). As described in more detail elsewhere, the clinical diagnosis of AD/Park was established by the coexistence of confirmed AD and clinical signs of parkinsonism, although good response to L-dopa was infrequent (Murray et al. 1995). The operating definition of AD/Park is based on the presence of AD pathology and absence of PD by pathologic criteria. Analysis of the substantia nigra with quantitative measure has identified only a minor loss of tyrosine hydroxylase positive neurons in AD/Park, which is of much smaller magnitude than in PD (Murray et al. 1995). Presence of cortical Lewy bodies was assessed in all cases and was identified in one PD case and non of the AD or AD/Park cases. The duration of symptoms was established in most cases, but the severity of the disorders were unknown. The duration of AD symptoms did not differ between the AD And AD/Park groups. The duration of parkinsonism did differ between the PD group and AD/Park group (Table 1). The degree of loss of DA uptake sites in the striatum of the majority of these cases was established and presented in detail elsewhere (Murray et al. 1995).

In all cases, following autopsy, the tissue (2 × 3 cm) was frozen rapidly in 2-methylbutane at a temperature of −40°C to ensure rapid freezing and stores at −70°C until processed for autoradiography. Coronal slabs corresponding to plates 7-9 and 11-25 of the atlas of Roberts and Hanaway (1971) were sectioned and processed for receptor autoradiography. The sections corresponding to plate 7-9 included head of caudate, rostral putamen, rostral globus pallidus, and nucleus accumbens. The sections corresponding to plate 13-25 included caudate, putamen, globus pallidus internal (GP_i), and globus pallidus external (GP_e). In all control and disease cases, the coronal levels were matched for their rostral-caudal position. For autoradiographic experiments, the

Table 1. Clinical Characteristics of the Cases

Group	Age	Sex	PMI	Medication	Symptoms[a]
CO					
1	77	M	12		
2	68	F	11		
3	73	F	4		
4	66	M	14		
5	70	F	7		
6	71	M	15		
7	64	F	18		
8	80	F	3		
9	78	M	5		
10	85	F	11		
11	56	M	4.5		
12	52	F	4.5		
13	67	F	5.5		
14	67	M	12		
Mean	70 yr		9.0 hr		
SD	±9		±5		
AD					
15	80	F	16		6 yrs AD
16	56	F	5	NA	6 yrs AD
17	82	M	14		10 yrs AD
18	77	F	4		3 yrs AD
19	64	M	3.5		14 yrs AD
20	76	M	8.5		3 yrs AD
21	68	M	8		5 yrs AD
22	77	F	3		10 yrs AD
23	84	M	16		5 yrs AD
Mean	76 yr		9 hr		7
SD	±9		±5		±4
PD					
24	71	M	3.5	NA	NA
25	77	F	6	S = 9 yrs	12 yrs PD
26	67	M	12	S = 16 yrs	16 yrs PD
27	74	M	2	S = 19 yrs	22 yrs PD
28	65	F	15	S = 10 yrs	13 yrs PD
29[b]	86	F	6	S = 11 yrs	15 yrs PD
Mean	73 yr		7 hr		16 yrs PD
SD	±8		±5		±4
AD/Park					
30	71	F	4	S = 6 mos	3 AD/3 Park
31	71	M	21	NA	NA/9 Park
32	74	F	3	S = 4 yrs	10 AD/7 Park
33	69	M	4.5	NA	NA
34	65	F	16	S = 3 yrs	11 AD/5 Park
35	80	F	15	S = 8 mos	8 AD/2 Park
	88	F	12	S = 28 mos	3 AD/3 Park
Mean	70 yr		10 hr		7 AD/5 Park
SD	±3		±8		±4 AD/3 Park

[a]Duration of illness in years. For the AD/Park cases, the duration for AD and parkinsonian symptoms (Park) are given seperately. NA, information not available.

[b]Case diagnosed at autopsy with cortical Lewy bodies.

Postmortem interval (PMI) is the time between death and brain tissue acquisition.

Medication: S = treatment with Sinemet; none of the cases were treated with antipsychotic.

Abbreviations: AD = Alzheimer's disease, PD = Parkinson's disease, AD/Park = AD with coexistent parkinsonian symptoms but not PD pathology.

brains were sectioned at 20 μm in a Lipshaw 1800-N cryotome at −15°C, thaw-mounted onto gelatin subbed slides, dried at 0°C under reduced pressure, and then stored at −70°C until further use.

Quantitative Autoradiography Studies

[^{3}H]SCH 23390 was obtained from Dupont, New England Nuclear (Boston, MA, USA). [^{125}I]epidepride was a generous gift from Dr. Kim Neve of Oregon Health Sciences University. (+)-Butaclomal was purchased at RBI Ltd. (Wayland, MA, USA) and idazoxan (Imperial Chemical Industries, Wilmslow, Cheshire, UK) was a generous gift. All other reagents were manufactured by Sigma Chemical (St. Louis, MO, USA).

D_1 receptors were labeled using [^{3}H]SCH 23390 according to the methods previously published for human brain (Joyce et al. 1988). Slide-mounted sections were brought to 0°C 30 min prior to incubation. Slides were then preincubated for 5 min in buffer at room temperature (50 mm Tris, 120 mm NaCl, 5 mm KCl, 2 mm $CaCl_2$, 1 mm $MgCl_2$, pH 7.6). Incubations containing 2 nm [^{3}H]SCH 23390 were carried out for 60 min at room temperature in buffer containing 1 μm Mianserin (to block binding to 5-HT_{1C} and 5-HT_2 receptors), 1 mm ascorbic acid, and 10% w/v bovine serum albumin (BSA). Sections were rinsed 1 × 30 s in ice-cold preincubation buffer, followed by a dip in ddH_2O at 4°C and dried on low heat plate under a stream of cold air. Specific binding was defined as the binding displaced by 5 μm (+)-butaclomal.

D_2 receptors were labeled using [^{125}I]epidepride according to the method previously established for human tissue (Joyce et al. 1991). Slide-mounted sections were brought to −20°C 30 min prior to incubation and subsequently dried. Side-mounted sections were preincubated for 30 min in ice-cold TBS (50 mm Tris-HCl, 120 mm NaCl, pH 7.4) and then for 25 min in TBS at room temperature. Slide-mounted tissue sections were incubated in the buffer solution containing 80 to 100 pm [^{125}I]epidepride (2200 Ci/mmol) for 60 min at 30°C in TBS containing 100 nm idazoxan (to block binding to $alpha_2$ adrenergic receptors). Specific binding was defined as the binding displaced by 5 μm (+)-butaclomal. Slides were rinsed 4 × 7 s each in ice-cold TBS followed by a dip in ddH_2O at 4°C and then dried under a stream of cold air.

The dried slide-mounted tissue sections and standards (ARC, Inc.) were loaded into light-proof x-ray cassettes and apposed to tritium-sensitive film (Amersham Hyperfilm). The film was exposed for 18 h for [^{125}I]epidepride and 6 weeks for [^{3}H]SCH 23390. The films were then developed with Kodak GBX developer at 15°C for 5 min and fixed with Kodak GBX fixer at 15°C for 5 min. Analysis of the autoradiographs was carried out using a computer-based image analysis sys-

tem. The illuminated image of each autoradiograph was collected by a video camera connected to an IBM-based image analysis system (DUMAS, Drexel University, Philadelphia, PA, USA). This system enabled the autoradiographic images to be digitized, and the mean optical density of each brain region of interest was converted to a value of fmol/mg protein. For autoradiographs obtained by sections labeled with a ^{3}H-ligand, a standard curve for ^{3}H based on calibrations of low activity ^{3}H-plastic standards to ^{3}H-isoleucine-tissue mash standards was used (Joyce et al. 1986a). For autoradiographs obtained by sections labeled with a ^{125}-ligand, standards calibrated against ^{125}I-tissue mash standards for the appropriate exposure time was used according to the method described by Artymyshyn et al. (1990). This allowed for the transformation of the autoradiograph so that the gray values of each pixel are a linear function of the quantity of radioligand bound per milligram of protein.

Data Analysis and Statistics

The striatum was divided into four levels in the rostral-caudal axis for the analysis of the material (Murray et al. 1995). For each level, a minimum of four sections for total binding and two sections for nonspecific binding sections were analyzed to provide a mean value for each case for each level. Average values were pooled for all cases by region and disease. In an attempt to determine whether a heterogeneous pattern of binding exists in the confirmed diseases as compared to control, a two-dimensional subregion analysis was carried out encompassing both the rostrocaudal and dorsoventral gradients throughout the basal ganglia. However, initial analysis by ANOVA with regions as repeated measure indicated that dividing the caudate and putamen into a dorsal and ventral division did not provide a reduction in the error term, and the divisions were combined for further analysis. General differences in binding between control and disease cases (PD, AD, and AD/Park) were determined using one- and two-factor ANOVA. Post-hoc paired comparisons were tested for the significance of difference using the method of Student–Newman Keuls (SNK, $p < .05$).

RESULTS

D_2 Dopamine Receptors Labeled with [^{125}I]epidepride

The ratio of total to nonspecific binding was 9.9 to 1 for [^{125}I]epidepride. A two-factor ANOVA showed a statistically significant main effect of diagnosis (control, AD, PD, AD/Park; $p = .023$). Also, differences in number of [^{125}I]epidepride binding sites between rostral and caudal levels were significant only for the control group (repeated measures; $p = .0001$, interaction $p = .4$) for sites in caudate and putamen (Figure 2B,D). As described previously (Joyce et al. 1991), the control group showed a greater number of [^{125}I]epidepride binding sites in the rostral caudate as compared to the caudal caudate by 27%, but the rostral-caudal gradient was absent for AD, PD and AD/Park groups. One-factor ANOVA showed a statistically significant effect of diagnosis for the caudate nucleus ($p < .05$). The AD/Park (Figures 1D, 2B) showed significantly less binding than the control group (reduced 42%; Figure 1A), PD group (reduced by 41%; Figure 1C), and AD group (reduced 54%; Figure 1C, 2B). In the nucleus accumbens, the concentrations of [^{125}I]epidepride binding sites did not differ between groups.

A two-factor ANOVA showed a statistically significant main effect of diagnosis (control, AD, PD, AD/Park; $p = .023$). The putamen of the control group displayed an average value of 100 ± 7.5 fmol/mg protein [^{125}I]epidepride binding to D_2 receptors. The control group showed a higher number of [^{125}I]epidepride binding to D_2 receptors in the rostral putamen as compared to the caudal putamen by 34%, but the rostral-caudal gradient was absent for AD, PD, and AD/Park groups. The AD/Park group exhibited significant but smaller reductions in [^{125}I]epidepride binding sites as compared to effects in the caudate, with a 27% reduction in D_2-like receptors as compared to the control group (Figure 2C) and the AD group (reduced by 55%) and nonsignificantly less binding than the PD group (reduced by 30%).

As described previously, [^{125}I]epidepride binding to D_2 receptors was higher in the GPe than the GPi (Joyce et al. 1991). A two-factor analysis of variance did not show a statistically significant main effect of diagnosis (control, AD, PD, AD/Park; $p = .4$) but it did show an effect of repeated measure ($p = .0001$) for GPe and GPi. In the GPe of the control group [^{125}I]epidepride binding to D_2 receptors showed a mean value of 29 ± 5 fmol/mg protein which is approximately 50% the number of receptors seen in striatum. In the GPi of the control group, [^{125}I]epidepride biding to D_2 receptors is 10% of the numbers (13.5 ± 4 fmol/mg protein) of D_2-like receptors seen in control striatum. There are no significant differences in receptor number for the PD (29 ± 6 fmol/mg protein), AD (37 ± 20 fmol/mg protein), or AD/PARK (15 ± 5 fmol/mg protein) groups as compared to the control group for the GPe.

D_1 Dopamine Receptors Labeled with [^{3}H]SCH 23390

The ratio of total to nonspecific binding was 5 to 1 for [^{3}H]SCH 23390 binding. Significant differences by rostral and caudal levels did not exist and were collapsed for further analysis. Significant changes in receptor

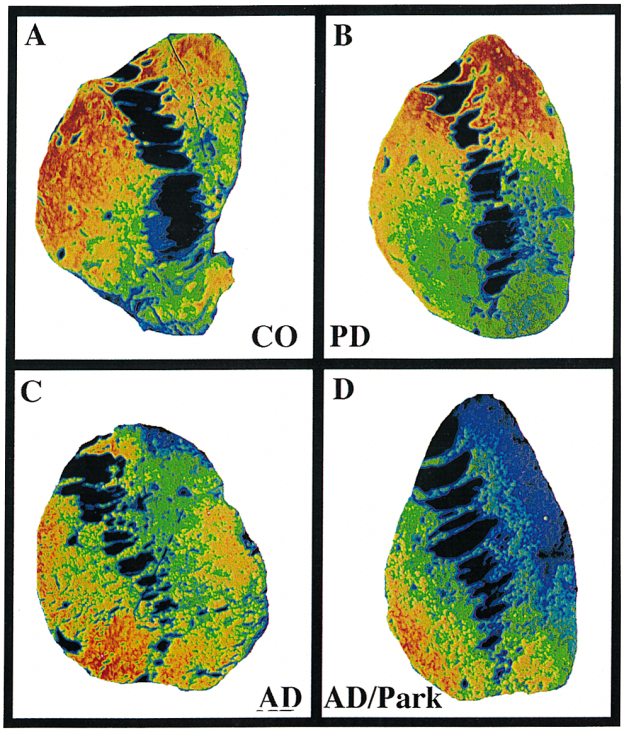

Figure 1. Pseudocolor representation of the original autoradiographs for binding of [^{125}I]epidepride to D_2-like receptors in rostral striatum. **(A)** a representative control case (CO). **(B)** a representative Parkinson's disease case (PD). **(C)** a representative Alzheimer's disease case (AD). **(D)** a representative Alzheimer's with coexistent parkinsonism case (AD/Park). For pseudocolor, red represents maximum density of sites and blue the lowest density of sites.

numbers from the control group was observed for the PD group ($p = .01$). In the putamen, the control group did not differ in D_1 receptor number from the AD or AD/Park groups (Figures 2C, 3). The PD group exhibited 36% more sites than the control group, 69% more than the AD/Park, and 158% more than the AD group ($p < .05$ SNK). There were nonsignificant decreases in the caudate nucleus for the AD group (by 19%) and a nonsignificant elevation in D_1 receptor number for the PD group (Figure 2A). In the nucleus accumbens, the control group did not exhibit significant differences in receptor number from any of the groups.

Initial analysis GPe and GPi by ANOVA with regions as repeated measure indicated that dividing the globus pallidus into external and internal segments provided a reduction in the error term only for the control group. A two-factor ANOVA did not show a statistically significant main effect of diagnosis (control, AD, PD, AD/PD; $p = .4$), but it did show an effect of repeated measure ($p = .0001$) for GPe and GPi. The GPe of the control group exhibited 68 ± 8 fmol/mg protein of [^{3}H]SCH 23390 binding to D_1 receptors, which was approximately 35% of the number of receptors seen in the striatum. The GPi of the control group demonstrated 43% of the numbers of D_1 receptors seen in control striatum (82 ± 17 fmol/mg protein) (Figure 3). The AD group (75 ± 20 fmol/mg protein), PD group (73 ± 25 fmol/mg protein), and AD/PD group (84 ± 16 fmol/mg protein) demonstrated similar values for the GP.

DISCUSSION

Our results indicate that, in addition to alterations in the presynaptic DA system (Joyce et al. 1997; Murray et al. 1995), the AD/Park group exhibited a loss of striatal D_2 receptors. Because this was not observed in AD lacking overt parkinsonian symptomatology or the PD group, it may contribute to the presence of parkinsonian features and poor responsiveness of L-dopa. Although [^{125}I]epidepride nonselectively labels two members of the D2-like receptor family, D_3 and D_2 receptors, with equally high affinity (Murray et al. 1995), the region where the loss of [^{125}I]epidepride biding was observed exhibits very low expression of the D_3 receptor (Joyce and Meador-Woodruff 1997; Murray et al. 1995). Hence, it is likely we are identifying selective changes in the binding to D_2 receptors. Furthermore, D_2-like receptor binding was reduced in the AD/Park group with no changes in D_1 receptor number. Additionally, in the AD cases not exhibiting parkinsonian symptoms, a nonsignificant increase, not a decrease, in [^{125}I]epidepride binding to D_2 receptors was observed. This would suggest that there is a specific disorder modifying the expression of D_2 receptors or the neurons expressing this receptor in the AD/Park group.

Previous autopsy studies have found that there is a reduction of D_2 receptors using [^{3}H]spiroperidol in the striatum of AD but those studies did not differentiate between those exhibiting parkinsonian symptoms and

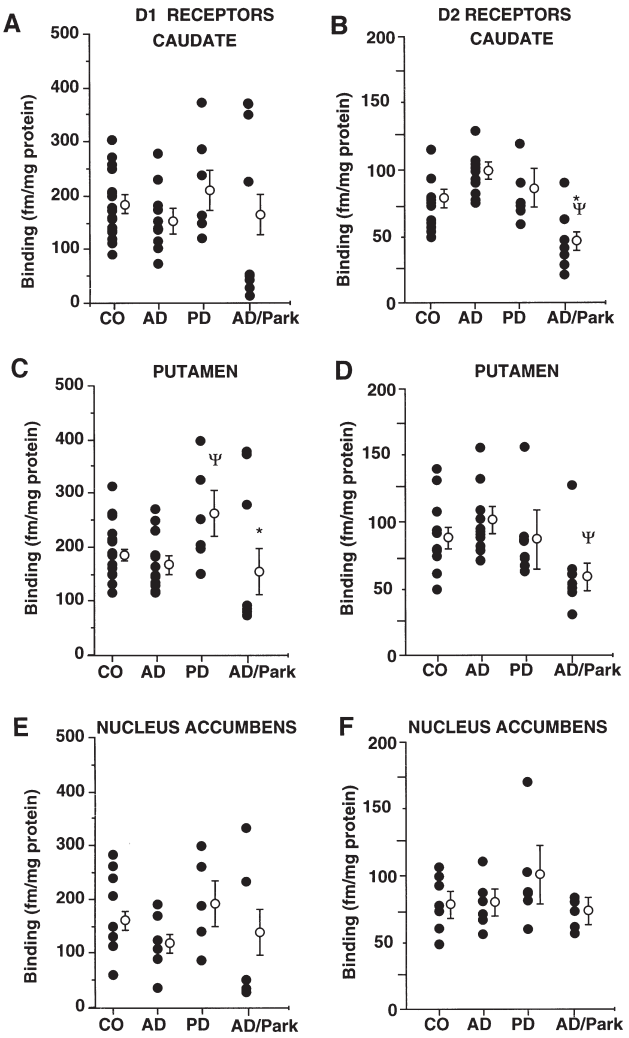

Figure 2. Scatter graphs representing numbers of D_1 receptors **(A, C, E)** or D_2 receptors **(B, D, F)** in regions of striatum for control (CO), Alzheimer's disease (AD), Parkinson's disease (PD) and Alzheimer's with coexistent parkinsonism (AD/Park). D_1 receptors labeled with [^{3}H]SCH 23390 are shown for caudate **(A)**, putamen **(C)**, and nucleus accumbens **(E)**. D_2 receptors labeled with [^{125}I]epidepride are shown for caudate **(B)**, putamen **(D)**, and nucleus accumbens **(F)**. For graphs all graphs Ψ, $p < .02$ significantly different from control and AD; $^*p < .05$ significantly different from PD. The open circles represent the mean value ± SEM for each group, individual values are represented by the closed circles.

those without (Cortés et al. 1988; Cross et al. 1984; Reisine et al. 1978; Rinne et al. 1986). In addition the results of a recent SPET study indicated to the authors that a loss of D_2 receptors could contribute to the parkinsonian features that were apparent in a number of their AD Cases, but they did not observe any differentiation between those exhibiting or not exhibiting parkinsonian symptoms (Pizzolato et al. 1996). Our results differ from the previous studies in that we did observe differences in the two AD groups. We believe that the decline in the number of postsynaptic striatal D_2 receptors could be one of the characteristic pathologic changes of AD/Park, that distinguish it from AD without parkinsonian features. Our results also suggest that, along with changes in the ability to process DA appropriately (Joyce et al. 1997; Murray et al. 1995), the loss of D_2 receptors in those AD cases exhibiting parkinsonian symptoms might explain the incomplete response of these patients to L-dopa treatment (Merello et al. 1994; Duret et al. 1989).

In this study, we also found that the PD group showed an increase in D_1 receptor number in the putamen but no elevation in D_2 receptor number. The increase in D_1 receptors may reflect changes in one subpopulation of the cases. For example, the scatter graph of the data indicates that there are two groups of PD cases, those with high numbers and those with low number of D_1 receptors. Previous studies of striatal D_1 receptor expression in PD cases have often shown increases in receptor number, but significant variability is observed, suggesting a bimodal population (Joyce 1993; Seeman et al. 1987; Rinne et al. 1985). One possibility for this is that treatment with L-dopa in the PD group added to the variability of the results, because it seems to modify D_1 receptor number (Seeman et al; Rinne et al. 1985). In MPTP-treated monkeys, we have observed an increase in D_1 receptor number that is enhanced by chronic administration of L-dopa leading to abnormal involuntary movements (Rioux et al. 1997). The pattern of DA fiber loss in PD is closely mimicked by administration of MPTP in monkeys to make them parkinsonian (Joyce et al. 1986b). The animals also develop abnormal involuntary movements similar to what happens in patients with advanced PD in response to L-dopa. Hence, the increase in D_1 receptors may contribute to the unwanted extrapyramidal side-effects of L-dopa treatment that occurs in some advanced PD cases.

Although we (Joyce 1993) and others (Bokobza et al. 1984; Seeman et al. 1987) have observed increases in [^{3}H]spiroperidol binding to D_2 receptors in PD, we did not find an elevation in number in this study using [^{125}I]epidepride to label D_2-like receptors. We have previously observed that D_2-like receptors labeled with [^{3}H]spiroperidol are increased in the lateral putamen of PD cases and in the dorsal putamen and dorsal caudate of MPTP-treated monkeys (Joyce 1993; Joyce et al. 1986b). In rat and monkey models of parkinsonism, there is evidence that the topographically restricted change in D2 receptor number is a reflection of both the pattern of DA loss and properties of the neurons in that region of the striatum (Joyce 1991; Joyce et al. 1986b). A greater than 70% loss of DA innervation to the region is required before D_2-like receptor number is increased. Although a greater than 90% loss of [^{3}H]mazindol binding to DA terminals occurred in the putamen and greater than 80% losses occurred in the caudate of these

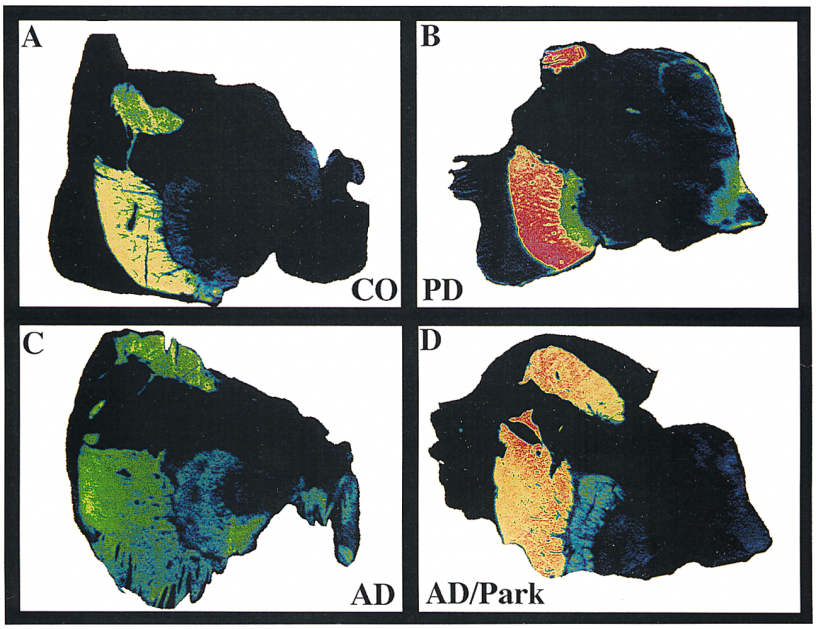

Figure 3. Pseudocolor representation of the original autoradiographs for binding of [^{3}H]SCH 23390 to D_1 in caudal striatum. **(A)** a representative control case (CO). **(B)** a representative Parkinson's disease case (PD). **(C)** a representative Alzheimer's disease case (AD). **(D)** a representative Alzheimer's with coexistent parkinsonism case (AD/Park). For pseudocolor, red represents maximum density of sites and blue the lowest density of sites.

cases (see Murray et al. 1995), we did not observe an increase in D_2 receptors labeled with [^{125}I]epidepride in this study of PD cases. Although antemortem drug conditions could effect the results; for example, L-dopa is thought to down-regulate D_2 receptors (Guttman et al. 1986; Pizzolato et al. 1995; Rinne et al. 1983), other factors may have contributed to the differences in the results. The D2 receptor family includes three subtypes, names D_2, D_3, and D_4, (Sibley et al. 1993), which exhibit distinct pharmacological properties and are concentrated in human brain in different regions (Joyce and Meador-Woodruff 1997). [^{125}I]epidepride nonselectively labels D_3 and D_2 receptors with equally high affinity and have very low affinity for the D_4 receptor (Murray et al. 1994); whereas [^{3}H]spiroperidol has low affinity for the D_3 receptor (Sokoloff et al. 1990). It is now clear that D_2-like receptors labeled with [^{3}H]spiroperidol (Joyce et al. 1986a) have a distribution different from that of D_2 and D_3 receptors labeled with [^{125}I]epidepride in striatum. (Joyce et al. 1991; Murray et al. 1994). Studies in the rat indicate D_3 receptors may be regulated by loss of DA afferents differently from the D_2 receptor and produce a reduction in number (Lévesque et al. 1995). We have recently demonstrated that striatal DA loss in PD also alters D_3 receptors in a manner opposing that of D_2 receptors and, thus, make it difficult to differentiate between these effects in this study (Ryoo et al. 1998). However, the region in which we have previously observed changes in D_2 receptor (dorsal putamen) number exhibits very low expression of the D_3 receptor (Joyce et al. 1991; Murray et al. 1994). Hence, it is likely we are predominantly labeling D_2 receptors in that region. This would suggest that the failure to identify an elevation in D_2 receptor number in this study may be attributable to other factors. A more detailed analysis of the response of D_2 and D_3 receptors to loss of DA innervation in PD is warranted.

Our data suggest that the regulation of DA receptors differs between PD, AD, and AD cases exhibiting parkinsonism. The mechanisms responsible for the loss of D_2-like receptors in AD/Park is unclear. Numerous amyloid deposits have been identified in the striatum in AD, particularly in those with parkinsonian features, and this may contribute to altered processing of proteins in these cells (e.g., receptors) (Braak and Braak 1990). Consequently, the ability of striatal neurons to synthesize D_2 receptor protein and transport these proteins to the appropriate synapses may be significantly affected (see Joyce et al. 1988). However, it is unclear why D_2-like receptors would be reduced in number when other DA (e.g., D_1) receptors expressed in the striatum were not. The majority of AD/Park cases were provided L-dopa treatment when PD symptoms were observed but for relatively short periods of time. Thus, down-regulation of the D_2-like receptor is not likely to have occurred. There is the possibility that the AD/Park cases had neuroleptic treatment during some part of the course of their illness that we did not identify in our prospective review of their records. The combination of the effects of these drugs on expression of D_2-like receptors is not known and might represent another potential source of variability. However, we are relatively certain that the cases did not receive neuroleptics during the course of their illness. For that reason, we are also reasonably certain that the reduction in D_2-like receptors does not reflect competition by neuroleptics

for the binding of [^{125}I]epidepride. This would suggest that there is a disturbance in the ability of a specific population of neurons to express D_2 receptors in the AD/Park group (Le Moine and Bloch 1995). Similar suggestions have been made for multiple system atrophy in which *in vivo* imaging techniques (PET and SPET) have demonstrated a reduction in D_2-like receptor binding (Antonini et al. 1997; Schulz et al. 1994). We believe that the data add to our previous results showing that presynaptic components of the DA system are modified in AD cases exhibiting parkinsonism, and this differs from either AD or PD (Joyce et al. 1997; Murray et al. 1995). The contribution of the loss of D_2 receptors to the parkinsonian symptoms in AD and lack of response to L-dopa should be explored further.

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