antigen in the context of class II MHC molecules.

It is not yet possible to know whether a functionally significant amino acid change of the type reported here mediates its effect by altering the overall conformation of the I-A molecule, by changing the local tertiary structure of I-A_β, or by substituting a new side chain directly at a site physically involved in T-cell receptor-antigen-Ia interaction. Nonetheless, it is clear that even a nonconservative change in a normally invariant residue (Glu at I-A^k_β position 67; see Fig. 2B) does not destroy all immunologic activity of the resultant I-A, since certain anti-A^k_β monoclonal antibodies and I-A^k–restricted T cells still recognize such molecules. These findings suggest that this mutation does not change the gross three-dimensional structure of the I-A molecule. If so, then one may speculate that the loss by A19/B13 of antigen-presenting function to HEL-C10 is related to the local alteration of a site directly involved in either binding by the T-cell receptor (the so-called "histotope") or interaction with HEL-derived "processed" antigen (the "desetope") (*24*). Further experiments are required to distinguish between these possibilities.

The approach taken here cannot map all potentially important portions of I-A molecules, since it is constrained by the serologic reagents used and the panel of T hybridomas tested. However, it serves as an important complementary approach to site-directed mutagenesis and in vitro recombination. Used together, these techniques should continue to yield important insights into the structural basis for MHC-restricted T-cell recognition of antigen.

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Quantitative Analysis of D2 Dopamine Receptor Binding in the Living Human Brain by PET

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D2 dopamine receptors in the putamen of living human subjects were characterized by using the selective, high-affinity D2 dopamine receptor antagonist carbon-11–labeled raclopride and positron emission tomography. Experiments in four healthy men demonstrated saturability of [^{11}C]raclopride binding to an apparently homogeneous population of sites with Hill coefficients close to unity. In the normal putamen, maximum binding ranged from 12 to 17 picomoles per cubic centimeter and dissociation constants from 3.4 to 4.7 nanomolar. Maximum binding for human putamen at autopsy was 15 picomoles per cubic centimeter. Studies of [^{11}C]raclopride binding indicate that clinically effective doses of chemically distinct neuroleptic drugs result in 85 to 90 percent occupancy of D2 dopamine receptors in the putamen of schizophrenic patients.

BINDING OF LABELED LIGANDS TO neurotransmitter receptors in the living human brain has been demonstrated with positron emission tomography (PET) (*1–3*). Models have been proposed for the measurement of binding variables in PET-scan studies, but their applicability has been limited by the slow achievement of equilibrium with available ligands (*4–7*). Receptor densities and affinity characteristics have therefore not been determined for any neurotransmitter receptor system in the living human brain. In vitro methods have supplied evidence for increased densities of D2 dopamine receptors in the dopamine-rich basal ganglia of deceased schizophrenics (*8–11*). A quantitative in vivo method would help to clarify the importance of these alterations in the pathophysiology of schizophrenic disorders.

We previously described the usefulness of a new ligand, [^{11}C]raclopride, for PET-scan studies of D2 dopamine receptors in the human brain (*3*). Unlike other ligands used for such studies, raclopride has a high selec-

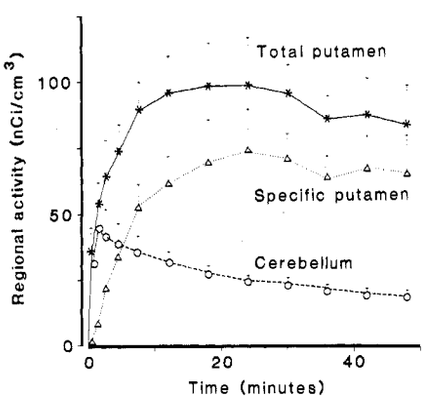

Fig. 1. Radioactivity in the putamen and cerebellum in six healthy men after intravenous injection of [^{11}C]raclopride (2.7 mCi). The radioactivity was observed sequentially for 1- to 6-minute periods during 50 minutes after the injection. The radioactivity in the cerebellum was assumed to reflect free and nonspecific binding. Specific binding in the putamen was calculated as the difference between radioactivity in the putamen and that in the cerebellum. Values are means ± standard deviations.

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tivity and affinity for central D2 dopamine receptors and negligible affinity for D1 dopamine, S2 serotonin, and α_1 adrenergic receptors (*8, 9*). In this study we analyzed the saturation of [^{11}C]raclopride binding by varying the specific activity of this ligand in a series of experiments on four healthy men. The use of [^{11}C]raclopride is appropriate because it rapidly associates with D2 dopamine receptors and shows little nonspecific binding (*3*).

The blockade of D2 dopamine receptors in the brain by antipsychotic drugs has been related to both the side effects and the therapeutic antipsychotic effects of these compounds (*10*). Therefore we also examined the reduction of D2 dopamine receptor binding of [^{11}C]raclopride in schizophrenic patients treated with three chemically distinct classes of neuroleptics.

Six healthy male volunteers 26 to 28 years of age took part in the study; four of these subjects participated in the detailed analysis of saturation. Three male schizophrenics 42 to 51 years of age, who had been treated with various neuroleptics for at least 5 years and who had responded well to treatment, were also recruited. [^{11}C]Raclopride was synthesized and prepared as decribed by Ehrin *et al.* (*11*). For the saturation analysis a series of five experiments was performed on each healthy subject (*12*).

Specific binding was defined as the difference between radioactivity in the putamen and that in the cerebellum, since there is only slight binding to D2 dopamine receptors in the cerebellum (*13, 14*). The total radioligand concentration in the cerebellum provided an estimate of the concentration of "free" radioligand. The estimates of specific binding and free radioligand concentration were used in the hyperbolic function

$$\text{Bound} = \frac{B_{max} \times \text{free}}{K_d + \text{free}}$$

for the determination of B_{max} (maximum binding) and K_d (dissociation constant) values in the putamen of each subject. An iterative program (BMDP, developed at the University of California, Los Angeles) was used for these calculations. For comparison, Hill plots were generated. The B_{max} obtained for each individual was used in the linear regression analysis for obtaining a K_d value and the Hill coefficient.

The expected [^{11}C]raclopride binding in the putamen of the schizophrenic patients, had they not been undergoing drug treatment, was based on the measured free radioligand concentration in the cerebellum of each patient and the average B_{max} and K_d values for the healthy volunteers (Table 1). It was assumed that the healthy subjects and the schizophrenic patients had similar D2 dopamine receptor densities and binding affinities in the putamen. The relative receptor occupancy during drug treatment was expressed as the percent reduction of expected specific binding.

Specific binding of [^{11}C]raclopride in the putamen was maximal after about 24 minutes and declined only slightly thereafter (Fig. 1). Cerebellar radioactivity, reflecting predominantly the free ligand concentration, was maximal after 2 minutes, indicating rapid passage of [^{11}C]raclopride across the blood-brain barrier.

The values for specific binding and free radioligand concentrations at 42 minutes were selected for the saturation analysis. Figure 2 presents the saturation hyperbola for specific [^{11}C]raclopride binding in the putamen of each of four healthy men. As shown, the experimental data points were close to the data retrieval hyperbola curves for all the subjects. On the basis of the saturation hyperbolas B_{max} and K_d values were calculated (Table 1). Hill plots of the data for the four subjects gave Hill coefficients close to unity (0.97 to 1.05), indicating binding to a single class of binding sites (Fig. 3).

The schizophrenic patients were treated with haloperidol, sulpiride, and flupenthixol. When the drugs were given in clinically antipsychotic doses, the relative degree of [^{11}C]raclopride binding to D2 dopamine receptors indicated an 84 to 90 percent blockade of these receptors in the putamen (Table 2 and Fig. 4).

Neurotransmitter receptors in rodents have been studied in vitro and in vivo by autoradiography (*15*). In the living human brain, PET has been used for the qualitative visualization of the distribution of ligand binding to neurotransmitter receptors (*1–7*). We used the technique to quantitatively characterize D2 dopamine receptors in the putamen of healthy male volunteers. The validity of our method is based on several assumptions:

1) The total radioactivity in the brain during the experiment represents unchanged [^{11}C]raclopride. Köhler *et al.* (*16*) found that after the intravenous injection of [^{3}H]raclopride into rats, more than 90 percent of the radioactivity in the brain after 45 minutes represented the unchanged drug. Raclopride metabolism in man follows a similar pathway and proceeds at a lower rate than in rats (*17*).

2) Equilibrium is reached within 42 minutes, at which time measurements are made. This assumption is based on the fact that

Table 1. Specific binding of [^{11}C]raclopride to D2 dopamine receptors in the putamen of four healthy men. The specific binding in the putamen was defined as the difference between the radioactivity in the putamen and that in the cerebellum. The determinations of B_{max} and K_d were performed with nonlinear regression analysis of specifically bound versus free ligand (using the cerebellar radioctivity of the dose given intravenously to estimate free ligand concentration).

Subject	B_{max} (pmol/cm^3)	K_d (nM)
A	17.0	3.4
B	13.6	3.4
C	14.2	3.6
D	12.7	4.7
Mean ± standard deviation	14.4 ± 1.9	3.8 ± 0.6

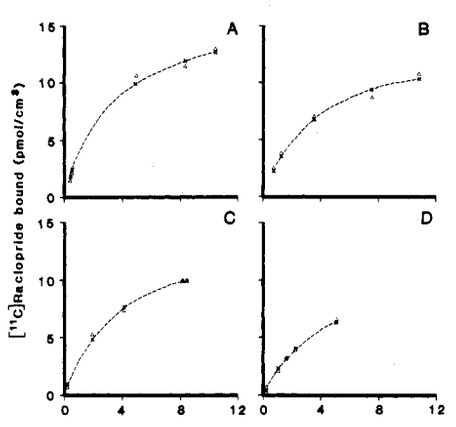

Fig. 2. Saturation curves for specific [^{11}C]raclopride binding to the putamen in four healthy men (A to D). The curves were obtained by nonlinear regression analysis with five experimental data points, each obtained with different doses (7.5 to 432 μg) of [^{11}C]raclopride (2.7 mCi). Specific binding was calculated as the difference between radioactivity in the putamen and that in the cerebellum. Binding in the cerebellum was assumed to reflect the free radioligand concentration. Triangles represent the experimental data and dashed lines the fitted curves, which were determined with the iterative program BMDP.

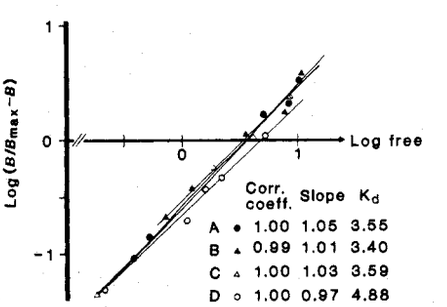

Fig. 3. Hill plots for the healthy subjects (A to D), generated with the same data as in Fig. 2. To calculate the Hill plot parameters the B_{max}value from the nonlinear regression analysis of the data for each individual was used. K_d values were determined from the intersection with the abscissa.

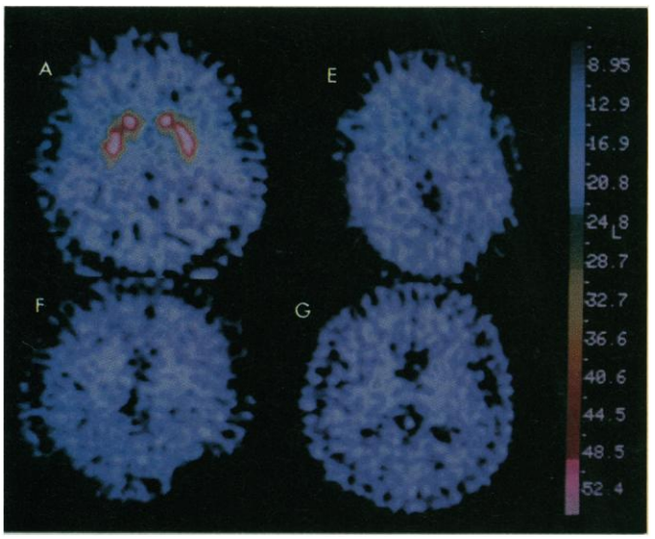

Fig. 4. PET scans through the caudate putamen of one healthy volunteer (subject A) and of three schizophrenics treated with haloperidol (4 mg orally twice daily) (E), sulpiride (400 mg orally twice daily) (F), or flupenthixol decanoate (100 mg intramuscularly weekly) (G). The scans show radioactivity accumulated during 10 to 51 minutes after the intravenous injection of [^{11}C]raclopride (2.7 mCi). The scale shows the relation between color and relative amount of radioactivity.

raclopride binds rapidly to D2 dopamine receptors (*3, 16*). K_d values were similar between 36 and 42 minutes, indicating that equilibrium had been reached. There is also a rapid establishment of steady state over the blood-brain barrier since the ratio of [^{11}C]raclopride concentration in plasma to concentration in the cerebellum remained constant after the first 2 minutes (*3*).

3) The radioactivity in the cerebellum reflects the free concentration of [^{11}C]raclopride throughout the brain and can be considered to consist of nonspecifically bound drug, free radioligand, and ligand within the regional vasculature. In binding assays in vitro, the nonspecific binding of [^{3}H]raclopride is approximately 5 percent of that specifically bound in the striatum at K_d (*16*). The concentration of [^{11}C]raclopride in the cerebellum was approximately 50 percent of that in the putamen at half-saturation in the present PET-scan study. Assuming similar percentages in rats and humans in vitro and in vivo, approximately 10 percent of the radioactivity in the cerebellum should be referred to nonspecific binding. The blood volume of the cerebellum is 3 to 4 percent (*18*), and the radioligand concentration in the blood is only slightly higher than that in the same volume of brain tissue. It can, accordingly, be estimated that 80 to 90 percent of the radioactivity in the cerebellum consists of free radioligand.

4) Release of endogenous dopamine does not significantly influence the binding of [^{11}C]raclopride to central D2 dopamine receptors. The affinity of dopamine for neuroleptic binding receptors is in the micromolar range, which is about three orders of magnitude lower than that for raclopride (*8, 19*).

Seeman *et al.* (*20*) found a B_{max} value of about 13 pmol/g for [^{3}H]spiperone binding to putamen from deceased human subjects. Moreover, [^{3}H]raclopride binding to putamen from deceased humans gave values of about 15 pmol/g (*21*), in excellent agreement with our results in vivo. This supports the validity of our in vivo approach to estimating D2 dopamine receptor densities in the human brain. The K_d values were also similar to those found for [^{3}H]raclopride binding to the human putamen in vitro (*21*), which further supports using the cerebellar concentration as the estimate for free ligand.

The results for the patients treated with different neuroleptic drugs indicate that many D2 dopamine receptors in the putamen of schizophrenics are blocked by clinically effective doses of antipsychotic drugs. Age and decease factors, which may have slightly influenced the results (but in opposite directions), were not considered (*6, 20*). The fact that three chemically distinct neuroleptics produced the same degree of D2 receptor blockade suggests that such blockade is the basis of the antipsychotic effect of these drugs.

The hyperbolic form of the saturation curves for [^{11}C]raclopride binding (Fig. 2) implies that a substantial increase in the doses of antipsychotic drugs is required to obtain a small increase in receptor occupancy above 85 percent. This may partly explain the great variation in the doses of neuroleptics recommended for schizophrenics. Detailed analysis of the quantitative relation between D2 dopamine receptor occupancy and the antipsychotic effects of different neuroleptics may lead to more precise methods for determining the optimum doses of new and conventional antipsychotic compounds.

Table 2. Effects of neuroleptic drug treatment on [^{11}C]raclopride binding to D2 dopamine receptors in the putamen of three male schizophrenics. "Expected" specific binding was calculated from the mean B_{max} and K_d values for four healthy subjects (Table 1) and from the concentration of [^{11}C]raclopride in the cerebellum (free ligand concentration) in the drug-treated patients.

Item	Subject		
	E	F	G
Age (years)	42	51	46
Drug	Haloperidol	Sulpiride	Flupenthixol decanoate
Dose (mg)	4 (twice daily)	400 (twice daily)	100 (weekly)
Time after last administration (hours)	6	5	30
Concentration of raclopride in cerebellum (pmol/cm^3)	0.37	1.33	0.61
Specific binding of raclopride (pmol/cm^3)	0.13	0.60	0.27
Expected specific binding of raclopride (pmol/cm^3)	1.28	3.75	2.00
Receptor occupancy (%)	90	84	87

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12. In each experiment 2.7 mCi of [^{11}C]raclopride was administered intravenously. The specific activity was systematically varied between 2 and 116 Ci/mmol. Thus the dose of raclopride administered ranged from 7.5 to 432 μg. One experiment was performed on each schizophrenic subject. [^{11}C]Raclopride (2.7 mCi; total dose, 8 to 29 μg) was injected after the regular neuroleptic medication was administered (Table 2). The four-ring PET system (PC-384) at the Department of Neuroradiology of the Karolinska Hospital was used to measure the regional concentration of the labeled ligand in seven transverse sections of the brain (*17*). A level 3 mm above Monro's foramen identified by computerized tomography was chosen as the midpoint of section 4, the cross plane selected for studying the putamen by PET. The spatial resolution of the reconstructed images is 7.6 mm (full width at half-maximum). Each experiment comprised 11 to 12 sequential scans (1 to 6 minutes per scan) during a

period of 45 to 51 minutes. Regional activity was measured for each sequential scan, corrected for ^{11}C decay, and plotted against time. The values obtained were not corrected for partial volume effects. With the camera system used, the recovery coefficient for the putamen was on the order of 0.90 (*17*). The putamen was chosen for quantitative measurements since it has the highest density of D2 dopamine receptors and the largest extension of the basal ganglia (*19*).
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Alterations of *myc*, *myb*, and *ras*^{Ha} Proto-oncogenes in Cancers Are Frequent and Show Clinical Correlation

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Alterations of c-*myc*, c-*ras*^{Ha}, or c-*myb* oncogenes were found in more than one-third of human solid tumors. Amplification of c-*myc* occurred in advanced, widespread tumors or in aggressive primary tumors. Apparent allelic deletions of c-*ras*^{Ha} and c-*myb* can be correlated with progression and metastasis of carcinomas and sarcomas.

PROTO-ONCOGENES MAY BE ACTIVATED and contribute to neoplastic transformation of cells through point mutations, translocations, deletions, gene amplification, or other genetic mechanisms. Mutations have been found most often in proto-oncogenes of the *ras* family, assayed by the NIH-3T3 transformation system (*1*). Chromosomal translocations involving cellular oncogenes occur in chronic myelocytic leukemias and Burkitt's lymphomas (*2*). Deletion of one allele of c-*ras*^{Ha} has been found in some Wilm's tumors (*3*) and other sporadic tumors (*4*). Amplification of several cellular oncogenes, including c-*myc*, c-*myb*, c-*ras*^{Ki}, c-*ras*^{Ha}, c-*abl*, and c-*erb* B oncogenes and of N-*myc*, have been reported in a number of human cancer cell lines (*5–7*) and in a few fresh human tumors (*8–11*).

Since most analyses have been performed with cultured tumor cells, it has been unclear how frequently alterations of cellular oncogenes occur in vivo. Furthermore, the possibility that similar alterations occur in various normal tissues has not been formally excluded. To obtain information on these points we examined 101 fresh human malignant tumors and 3 benign tumors representing 21 different histologic types, including 71 carcinomas, 9 sarcomas, 18 leukemias, and 3 lymphomas (Table 1). In 72 instances it was possible to obtain simultaneously normal tissues from the same patients, and in 64 of these the normal tissues were homologous with the cancers (such as colon cancer and normal colonic mucosa). DNA from these 176 samples was analyzed for alterations in proto-oncogenes with Southern blot hybridization (*12*) and 11 probes hybridizing either with nine different cellular oncogenes or with human β-globin (Table 2)(*6, 13–22*). Alterations were frequently found in c-*myc*, c-*ras*^{Ha}, and c-*myb*, and rarely in c-*ras*^{Ki} in tumors in vivo (Table 1). In contrast, no alterations in c-*fos*, c-*fes*, c-*abl*, N-*ras*, or c-*mos* were observed in the same samples, and no abnormalities of any of the nine proto-oncogenes in DNA specimens from normal tissues. Oncogene alterations appeared to be correlated with tumor behavior.

Amplification of the c-*myc* oncogene has been observed in several human cancer cell lines (*5–7*) and fresh tumors (*8–10*). The DNA was digested with Eco RI and hybridized (*12*) with a c-*myc*–specific probe and a β-globin probe (Fig. 1A). The single-copy β-globin gene served as an internal control for the amount of DNA transferred to the filters, and was used to estimate the copy number of the c-*myc* oncogene in normal and tumor tissues. In all DNA's tested, the human c-*myc* probe detected a 12.5-kb Eco RI fragment that contained the whole germline or non-rearranged human c-*myc* gene sequence (*23, 24*). The human β-globin probe detected a 3.1-kb Eco RI fragment that is at the 3′ end of the β-globin gene (*22*). The intensity of the β-globin signal was similar among 176 samples of normal

Table 1. Alterations in proto-oncogenes in human cancers.

Tumors	No.	Ratio of number with alterations to number studied			
		Amplified c-*myc*	Deleted c-*ras*^{Ha}	Deleted c-*myb*	Amplified c-*ras*^{Ki}
All types	101	10/101 (10%)	6/33 (18%)	4/35 (11%)	1/101 (1%)
Epithelial* (carcinomas)	71	8/71 (11%)	5/29 (17%)	3/33 (9%)	1/71 (1%)
Sarcomas†	9	2/9 (22%)	1/4 (25%)	1/1 (100%)	0/9 (0)
Leukemia‡ and lymphomas	21	0/21 (0)		0/1 (0)	0/21 (0)
Benign§	3	0/3 (0)	0/3 (0)	0/1 (0)	0/3 (0)
Primary‖	64	7/64 (11%)	4/26 (15%)	3/27 (11%)	0/64 (0)
Metastatic‖	16	3/16 (19%)	2/7 (29%)	1/8 (13%)	1/16 (6%)
None (normal tissue)	72	0/72 (0)	0/36 (0)	0/35 (0)	0/72 (0)

*Numbers of individual tumors are squamous of head and neck (or), skin (7), lung (3), esophagus (1), stomach (9), colon-rectal (32), kidney (4), breast (10), ovary (5). †Osteogenic (2), chondrosarcoma (1), soft tissue sarcomas (6). ‡Chronic myelocytic leukemia (8), chronic lymphocytic leukemia (6), acute myelocytic leukemia (3), acute lymphocytic leukemia (1), malignant lymphoma (3). §Colonic polyp (1), desmoid tumor (2). ‖Excluding leukemias and lymphomas.

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