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The Estimated Density of D_2 Striatal Receptors in Schizophrenia

A Study with Positron Emission Tomography and ^{76}Br-Bromolisuride

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The striatal D_2 receptors of 19 untreated schizophrenics and 14 normal control subjects were investigated with PET and ^{76}Br-bromolisuride. The ratio of radioactivity in the striatum to that in the cerebellum was taken as an index of the striatal D_2 receptor density. There was no significant difference between the control and the schizophrenic groups, nor any difference between subgroups of patients defined by clinical type or course of illness, and no relationship between the striatum:cerebellum activity ratio and SANS or SAPS ratings of symptoms. Unlike in the controls, this ratio was not correlated with age in schizophrenics. This study suggests that there is no quantitative abnormality of striatal D_2 dopamine receptors in schizophrenia.

Although at present questioned (Crow, 1987), one of the main biological theories of schizophrenia is the dopamine hypothesis. Since a common property of the neuroleptics is to block the dopamine D_2 receptors *in vitro* with a good correlation with their therapeutic potency (Creese *et al*, 1976), there could be an overactivity of the dopamine system in schizophrenia. As there is no compelling evidence of increased activity of dopamine neurones either from studies of cerebrospinal fluid or from assessment of post-mortem brain samples for metabolites of dopamine (Post *et al*, 1975; Crow *et al*, 1984; Bowers *et al*, 1974), research has been more recently orientated towards the central dopamine receptors.

Higher than normal densities of D_2 dopamine receptors have been found in several post-mortem studies of schizophrenic brains (Seeman, 1987; but see Kornhuber *et al*, 1989), but recent findings from *in-vivo* studies of D_2 receptors performed with positron emission tomography (PET) are discrepant. The Johns Hopkins team has found marked increases in the number of D_2 receptors in schizophrenics (Wong *et al*, 1986), while other authors could not replicate this finding (Farde *et al*, 1987; Martinot *et al*, 1990). However, in our previous study of striatal D_2 receptors with PET and ^{76}Br-bromospiperone, the hypothesis was formulated of state-dependent fluctuations of the striatal D_2 receptor density, with slight elevation of the D_2 receptor density during the onset or the exacerbation of the psychotic symptoms, but with no such elevation during the chronic course of the illness (Martinot *et al*, 1990).

To confirm these findings, we used ^{76}Br-bromolisuride, a new radioligand of the D_2 receptors, developed in our laboratory. *In-vitro* and *in-vivo* binding and competition studies in laboratory animals demonstrated a high affinity ($K_d = 0.3$ nmol/l) and a high specificity of this radioligand for D_2 dopamine receptors (Mazière *et al*, 1986).

Method

In-patients at the psychiatric departments of three hospitals, Ste Anne, la Salpêtrière, and Bicêtre, were recruited during the first week of admission if they fulfilled the following inclusion criteria: age over 18 years; DSM–III diagnosis of schizophrenic disorder (American Psychiatric Association, 1980); drug-naive condition or neuroleptic treatment interrupted for at least six months; and in a clinical condition allowing the patient to go without neuroleptics for the week of evaluation.

Informed consent was obtained in all cases. The Ethics Committee of the French Atomic Energy Commission approved the protocol of the study.

Patients were independently evaluated by four psychiatrists (MLPM, MFP, JLM, BB). They observed and interviewed each patient, interviewed the patient's relatives, and, in most cases, the physician who referred the patient, and examined the hospital report if there was one.

The group of patients was compared with a normal control group recruited from the hospital staff. The controls were screened by means of a physical examination, laboratory tests conducted in the preventive medicine department, and an interview by a psychiatrist. None used medications nor had physical or mental problems.

For the patients, quantitative clinical evaluation was performed with the Scales for the Assessment of Positive and Negative Symptoms (SAPS and SANS) (Andreasen, 1982). Several values were computed from the composite item scores and from the 'global evaluation' item score of each subscale. There were: (a) the sum of the composite scores and the sum of the global evaluation scores for the SAPS and for the SANS; (b) the sum of the composite and global evaluation scores of each scale (total score of each scale); (c) the general psychopathological rating as the sum of the total SAPS score plus the total score of the SANS.

PET technique

The bromolisuride, an antagonist of D_2 receptors, labelled with bromine-76 (^{76}Br-Blis) was used to study the striatal D_2 receptors. It has several advantages over bromospiperone: its striatal uptake is twice that of bromospiperone and the selectivity of its binding to the D_2 receptors is better, as there is no apparent binding of Blis with serotonin receptors, which is not the case for the bromospiperone (Mazière *et al*, 1986). The preparation of the radioligand and the description of the method have been published elsewhere (Mazière *et al*, 1990).

The ^{76}Br-Blis was injected intravenously as a slow bolus (0.006–0.020 nmol/kg in the controls, and 0.006–0.040 in the patients ($t = 2.70$, d.f. = 31, NS)). The mean (s.d.) amount of radioactivity injected was 0.96 (0.30) mCi for the control subjects and 1.07 (0.28) mCi for the patients ($t = 1.22$, d.f. = 31, NS), with a specific activity of 1.23 (0.63) nCi/ml for the control subjects and of 1.23 (0.46) nCi/ml for the patients (NS).

The PET studies were performed with the LETI TTV 01 time-of-flight positron tomograph, which provides seven simultaneous slices (in-plane resolution and slice thickness of 13 mm). Each subject was examined at rest, with the head positioned in the head-holder by reference to a laser beam system, according to a standardised procedure described by Baron *et al* (1986).

Before the injection of the radioligand a transmission study was performed for subsequent attenuation correction. After injection of the tracer, an early image was obtained within 15 minutes. A second emission scan, lasting 30 minutes, began two hours after the injection.

The blood radioactivity was measured from a venous blood sample taken at the end of the second scan; the mean (s.d.) value of this sample, normalised according to the weight of the subjects, was 0.86 (0.16) for the control subjects and 0.90 (0.15) for the patients ($t = 0.48$, d.f. = 24, NS).

The fraction of ^{76}Br-Blis specifically bound to the D_2 striatal receptors was estimated by the striatum:cerebellum ratio obtained two hours after injection. The concentration of tracer in the cerebellum is a measure of the non-receptor-associated tracer in the brain, while the striatal concentration reflects both specific and non-specific binding. Therefore, the striatum:cerebellum radioactivity concentration ratio is a reliable index of the radioligand's specific binding in the striatum, and reflects the density/affinity of the striatal D_2 dopaminergic receptors (Baron *et al*, 1986).

A standardised protocol using a video display was used to obtain cerebellar and striatal radioactivity concentrations from regions of interest (ROIs). For the cerebellum, in the reconstructed image of the first acquisition (10 mm above the orbitomeatal line), an automated isocontour program draws a line joining the picture elements having a radioactive concentration less than 30% of the image maximum. Then two circular ROIs of 15.8 cm^2 are placed tangentially to this line and the anteroposterior axis. These ROIs are copied in the corresponding image of the second acquisition. Each time, the mean cerebellar radioactive concentration is calculated and compared with the injected dose.

The calculation of the striatal radioactive concentration is somewhat more intricate because this structure often appears astride two adjacent slices. Measuring the striatal radioactivity in only one of these slices would underestimate the concentration. On the other hand, adding the striatal radioactivities measured in the two slices would overestimate the concentration. Accordingly, a standardised protocol has been defined.

On the late image(s) displaying a striatal accumulation, an automated isocontour program delineates the picture elements having a radioactive concentration above 80% of the image maximum. These striatal ROIs are copied on the images of the adjacent 'external' (non-striatal) slices (lower and upper slices). Then the radioactive concentration in striata is calculated as being the concentration or the sum of the concentrations of striatal ROIs minus the mean of the radioactive concentrations in ROIs measured on the 'external' slices.

This protocol was validated with a hot-spot phantom equivalent to human brain (and striata), which was moved under the PET camera in 5 mm increments. In the image having the maximum activity, the mean (s.d.) recovery yield of the radioactive concentration was 63 (11)% of the actual concentration. With our method of calculation, taking into account the radioactivity of adjacent slices, this recovery yield was 101 (7)%.

Statistical analysis

The striatum:cerebellum ratios of the patients v. the controls, and of the drug-naive patients v. the other patients, were compared by means of *F*-tests. Thereafter, the patients were pooled in several subgroups according to the clinical type; comparisons between subgroups were performed using a one-way analysis of variance. Correlations between patients' striatum:cerebellum ratios and the various scores derived from the rating scales were assessed using the Spearman rank correlation statistic.

Results

Nineteen schizophrenic subjects met the inclusion criteria. There were 12 men, aged 18–28 years (mean (s.d.) = 22 (4) years) and seven women, aged 19–35 years (mean (s.d.) = 24 (6) years). Ten patients were drug-naive, and nine had not received neuroleptics for at least six months. During the days preceding the PET study, five patients received benzodiazepines, which are known not to interfere with D_2 receptors (Keller *et al*, 1976).

According to the DSM–III criteria, the distribution of the clinical types and of the course of the illness was as follows: disorganised, seven patients; undifferentiated, eight patients; paranoid, three patients; catatonic, one patient. The course of the illness was subchronic for seven patients, chronic with exacerbation for five patients, and chronic without exacerbation for seven patients.

The control group comprised 14 men of similar age (19–33 years; mean (s.d.) 23 (4) years).

The accessibility of the tracer was comparable in the controls and in the patients; the cerebellar radioactivity

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Table 1
Mean (s.d.) ratios of activity in the striatum to that in the cerebellum

Groups	Number of subjects	Striatum:cerebellum ratio	*F*-test	*P*
Normal controls	14	3.87 (0.38)		
Schizophrenics	19	4.04 (0.46)	1.27	0.26
Drug-naive schizophrenics	10	3.95 (0.55)		
Drug-free schizophrenics	9	4.13 (0.35)	1.04	0.36*
DSM-III clinical type				
disorganised	7	3.99 (0.48)		
undifferentiated	8	4.08 (0.57)		
paranoid	3	3.96 (0.22)		
catatonic	1	4.25	0.41	0.80*
DSM-III course of the illness				
subchronic	7	4.08 (0.58)		
chronic with acute exacerbation	5	4.23 (0.55)		
chronic	7	3.85 (0.17)	1.24	0.31*

*Probability for the *F*-test value in the comparison of subgroups of patients between them and with the normal control group.

values from the early scan, normalised according to the subjects' weight, were 1.32 (0.30) for the normal controls and 1.40 (0.24) for the patients ($F=0.76$, d.f. = 31, $P<0.40$).

The cerebellar radioactivities measured two hours after injection (expressed as a fraction of the injected dose $\times 10^{-5}$) were considered as a measure of the non-specific binding of the ^{76}Br-Blis, as the cerebellum is devoid of D_2 dopamine receptors. These values were not significantly different in the control subjects and in the patients (mean (s.d.) for control subjects = 0.95 (0.15), for patients = 1.07 (0.27); $F=2.48$, d.f. = 31, $P=0.12$).

The mean values of the striatum:cerebellum ratios are presented in Table 1. There were no significant differences when the overall schizophrenic group was compared with the normal controls, or when patients who had had neuroleptics were compared with those who had not, or when the DSM-III subgroups (clinical type and course of the illness) were compared with each other and with the normal controls. Also, there was no significant correlation between the striatum:cerebellum ratios and the various ratings on the SANS and SAPS.

The striatum:cerebellum ratio was significantly correlated with age in the control group ($r=-0.53$, d.f. = 12, $P<0.05$): the regression line of the striatum:cerebellum ratio to the age was $-0.048 \times \text{age} + 5$, indicating a 4% decrease in the value of the ratio per decade. However, there was no significant correlation between age and striatum: cerebellum ratio in the patient group ($r=0.11$, d.f. = 17, $P=0.64$); moreover, the slope of the regression line was positive (striatum:cerebellum ratio $= 0.021 \times \text{age} + 3.77$). Also, there was no significant difference in the comparison of the regression lines for the drug-naive and other patients (analysis of variance of regression coefficients overgroups: $P=0.41$).

Discussion

There was no significant difference in the striatum: cerebellum ratio (an index of the density/affinity of the striatal D_2 receptors) measured with ^{76}Br-Blis in 19 untreated schizophrenics compared with 14 normal control subjects.

This finding confirms the result of our previous study of D_2 striatal dopamine receptors with the ligand ^{76}Br-bromospiperone in 12 untreated schizophrenics (Martinot *et al*, 1990). It is also consistent with the findings of Mackay *et al* (1982), who did not find any modification of ^{3}H-spiperone binding in the caudate or acumbens nucleus in a post-mortem study of untreated schizophrenic brains. Also, our result concurs with the *in-vivo* PET measurement of central D_2 receptors with ^{11}C-methylspiperone (Herold *et al*, 1985; Wong *et al*, 1985) or with the ^{11}C-raclopride (Sedvall *et al*, 1986; Farde *et al*, 1987) in untreated schizophrenics. We failed to replicate the findings of Wong *et al* (1986), but a series of technical and clinical factors have been identified that could account for the discrepancies between that and the other studies (Andreasen *et al*, 1988).

On the whole, these results provide evidence that there is no increase of the D_2 striatal dopamine receptors associated with the diagnosis of schizophrenia.

As in our previous study with ^{76}Br-bromospiperone, there was no relationship between the clinical types of the illness, or the rating-scale scores, and the striatum:cerebellum ratios. We cannot

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confirm the hypothesis of variations in densities of central D_2 receptors with the course of the illness. In the present study, the mean striatum: cerebellum ratios of the patients whose symptoms had recently appeared or worsened (i.e. those diagnosed subchronic or chronic with exacerbation) are slightly higher than that of the chronic patients, but the difference does not reach significance.

However, there was an intriguing lack of correlation between the age of schizophrenics and the density of the D_2 striatal receptors estimated by the striatum:cerebellum ratio. The decrease of the D_2 receptor number with ageing has been well established in post-mortem studies of non-psychiatric subjects. In the normal controls of this study, the significant negative correlation found between activity ratio and age (a 4% decrease in the value of the ratio per decade) is congruent with the 2.2% decrease per decade of the D_2 receptors reported by Seeman *et al* (1987) in a post-mortem study of 247 subjects (aged 0–104 years). It also agrees with the decline found by Baron *et al* (1986) in 17 control subjects (aged 20–86 years; regression line -0.008 age $+2.39$), and with the significant negative correlation found in another sample of 12 normal controls (aged 18–58 years; regression line -0.013 age $+2.43$) (Martinot *et al*, 1990), both studies using ^{76}Br-bromospiperone as a ligand for the D_2 receptors.

The lack of correlation between age and the activity ratio in schizophrenics was also found in our earlier study of 12 schizophrenics (aged 21–52 years). This correlation did appear in the controls. This finding is not accounted for by previous neuroleptic treatment. Consequently, the absence of this correlation in the patients could be attributed to an as yet unknown time-dependent factor of the illness.

In conclusion, this study suggests that there is no quantitative abnormality of the striatal D_2 receptors in schizophrenia.

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