Dopaminergic D2 Receptor SPECT Imaging in Rett Syndrome: Increase of Specific Binding in Striatum

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A dopamine deficiency has been implicated in Rett syndrome, a progressive encephalopathy in girls that involves movement, tonus and cognitive disorders. To test the hypothesis that striatal D2 receptors increase in number in early stages of the disease, we measured the binding potential of ^{123}I-Iodolisuride, a specific D2 ligand, in eleven Rett children aged 4–15 yr (7.9 ± 3.5 yr) (mean ± s.d.) and eight control subjects aged 3.5–13 yr (8.1 ± 3.8 yr) who exhibited other neurological disorders. Regional cerebral blood flow (rCBF) was also measured with SPECT using ^{133}Xe. The binding potential for ^{123}I-ILIS and D2 receptors was significantly higher in Rett (0.45) than in controls (0.23) ($p < 0.01$). An increase in ^{123}I-ILIS binding due to increased rCBF in patients' striata was excluded. Our results are consistent with a higher density of D2 receptors in young patients suffering from Rett syndrome because of reduced dopaminergic neurotransmission.

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The response of dopamine receptors to loss of dopaminergic neurotransmission in the living human is not known with certainty. Classically, neuroreceptors are expected to multiply when the neurotransmitter disappears and the binding potential of specific ligands should increase. Studies of striatal D2 receptors in Parkinson's disease generally have not confirmed this expectation (*1*), but a recent evaluation showed that D2-receptor density increases in patients who do not receive dopa treatment (*2*).

Rett syndrome is a condition characterized by symptoms mimicking loss of dopaminergic neurotransmission, particularly movement, tone and cognitive disorders, which appear during the first two years in life (*3,4*). No drug has demonstrated any efficacy and patients usually do not receive any dopaminergic treatment. Clinical condition worsens with age resulting in death before adulthood in most cases. The most consistent abnormality is an underpigmentation of substantia nigra neurons (*5*), and low levels of biogenic amines have been found in the cerebrospinal fluid and the brain of some patients (*6,7*). A priori, we therefore expect dopamine D2 receptors to be elevated in untreated cases of this syndrome. Such elevation has not been found in vitro (*8*) or in vivo (*9*). However, the in vitro study necessarily examined a terminally severe case, and neuroreceptors may change after death. The in vivo study was exclusively dedicated to adult cases. To test the hypothesis of increased D2-receptor density in the striatum of patients with Rett syndrome at early stages of the disease, we studied the binding potential of a specific D2 ligand, ^{123}I-iodolisuride (ILIS) in a pediatric population.

METHODS AND MATERIALS

Patients

Eleven Rett patients aged 4 to 15 yr (7.9 ± 3.5 yr) (mean ± s.d.) were studied. Clinical diagnosis was made by the same physician according to international criteria (*4*). Six were epileptic but seizure free for at least 1 wk before study. None was taking any dopaminergic medication. Eight nonRett patients were also studied as controls, aged 3.5–13 yr (8.1 ± 3.8 yr) (mean ± s.d.). These subjects had other neurological illnesses but magnetic resonance imaging (MRI) was normal. Six were epileptics and all but one had been free of seizure for at least 1 wk; the remaining patient (#15) exhibited a seizure 24 hr before study. Six Rett patients and one control had severe microcephaly. All were studied with the informed consent of their parents and the approval of the Ethics Committee of the French Atomic Energy Commission (CEA). Patient data are illustrated in Table 1.

Patient Preparation

Each patient underwent cerebral MRI and single-photon emission computed tomography (SPECT) investigations on the same day. The MRI and SPECT examinations required rectal pentobarbital premedication (5 mg/kg) to prevent any head movement. Rett and nonRett patients received the same premedication administered 1 hr before SPECT examination. The SPECT protocol consisted of two successive scans: first a regional cerebral blood flow (rCBF) study using ^{133}Xe; and then a D2-receptor study using ^{123}I-ILIS, a specific D2-receptor antagonist (*10*). Sodium perchlorate was orally administrated beginning 2 days before ^{123}I-ILIS injection to prevent any uptake of free ^{123}I by the thyroid.

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TABLE 1
Clinical Data

Patients	Sex	Age (yr)	Clinical pathology	HC (SD)	AET
1	F	4.1	Rett	−2	no
2	F	4.2	Rett	−4	no
3	F	4.8	Rett	−4	no
4	F	6.7	Rett	−2	no
5	F	7.1	Rett	−4	VPA
6	F	7.7	Rett	−2	CBZ
7	F	7.8	Rett	−4	PB,CZP
8	F	7.9	Rett	−3	CBZ
9	F	8.2	Rett	−1	no
10	F	13.5	Rett	−5	PB,CBZ
11	F	14.9	Rett	−4	VPA
mean (s.d.)		7.9 (3.5)			
12	F	3.5	NP encephalopathy	−4	no
13	M	4.6	Partial epilepsy	nl	CBZ
14	F	4.9	Facial angioma	nl	no
15	M	6.2	Partial epilepsy	nl	PHT,GVG
16	F	9.4	Partial epilepsy	nl	CBZ,CZP
17	F	10.4	Partial epilepsy	nl	PB,PHT
18	M	12.8	Partial epilepsy	nl	VPA
19	F	12.9	NP encephalopathy	nl	CBZ
mean (s.d.)		8.1 (3.8)			

NP = nonprogressive encephalopathy; HC = head circumference; nl = normal; AET = anti epileptic treatment; VPA = valproate; CBZ = carbamazepine; PB = phenobarbital; CZP = clonazepam; PHT = phenytoin; GVG = gamma-vinyl GABA.

In Vivo Imaging

MRI was performed using a GE-MS MRMAX (0.5 Tesla) and T1 and T2* weighted gradient echo sequences. The bicommissural line (anterior commissure-posterior commissure) and the orbitomeatal (OM) line, as indicated by an external oil reference, were located on sagittal slices and drawn on film. Transaxial oblique slices parallel to the bicommissural line were generated. Striata were located on the MRI images and their axial level was noted to further identify the corresponding SPECT slice. Caudate and lenticular nuclei were outlined on the MRI slice where they had their maximal surface area. Striata were therefore delineated in two symmetrical regions of interest (ROI). SPECT was then performed using a Tomomatic 564 (Medimatic) camera providing five contiguous transaxial slices (slice thickness 20 mm, in-plane resolution 12 mm). Using MRI data, calculations were made to position the head so that the third slice included the striata. Regional cerebral blood flow was measured first, using a 74 MBq/kg dose of ^{133}Xe intravenously. This method provides absolute rCBF values but does not require any arterial blood sampling (*11*). The radiation dose to the lung (the target organ) was 2.5–4.5 mGy (*12*). One hour after completion of the ^{133}Xe study, ^{123}I-ILIS was injected intravenously at doses ranging from 44 MBq to 122 MBq (mean 5.2 MBq/kg). The calculated radiation dose was 0.007 mGy/MBq to the thyroid, 0.005 mGy/MBq to the whole body and less than 0.27 mGy/MBq to the intestine (the target organ) (*13*). Tomographic imaging was performed 2 hr after ^{123}I-ILIS injection and lasted from 10 to 20 min depending on brain radioactivity.

Image Processing

The striatal ROIs drawn on MR images were transferred to the corresponding SPECT ILIS and rCBF slices after they had been normalized to the brain size of SPECT slices (Fig. 1). The mean surface area of these ROIs was 2.7 cm^2. Iodine-123 activities and rCBF were calculated and expressed in mean value per pixel. The ^{123}I-ILIS binding potential was approximated by a binding index (BI) (*14*) calculated on each hemisphere as the striatum minus hemislice-to-hemislice radioactive equilibrium concentration ratio (Fig. 1). The striatal rCBF was normalized for the hemislice rCBF.

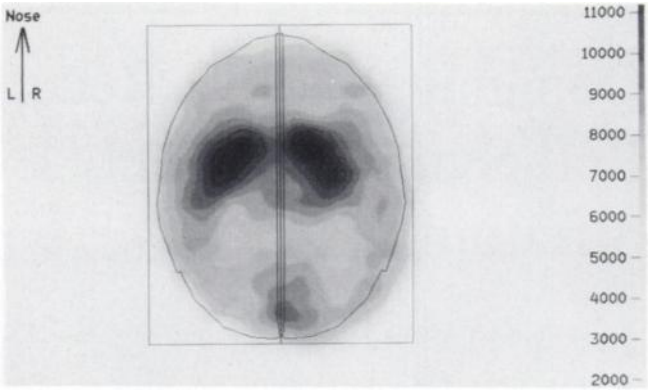

FIGURE 1. The striatal ROI is superimposed on the ILIS-SPECT image on the left and the right hemisphere. The corresponding hemislice is also delineated. Iodine-123 activity was measured in these four ROIs according to the scale reported on the image and an ^{123}I-ILIS BI was calculated from these data.

RESULTS

MR Images

All but two Rett patients (#4, 10) had moderate to severe subcortical atrophy. Seven Rett patients also had cortical atrophy which was severe in two cases (#3, 7). The non-Rett patient with microcephaly had severe corticosubcortical atrophy. MRI was normal in the other control children. Striata signal was normal in all 19 children.

SPECT Data

In all children, the striata were clearly visible in the images 2 hr after ^{123}I-ILIS injection (Fig. 2). The left and right values of BI and rCBF were not significantly different in Rett patients or in controls. Left and right values were therefore averaged. The striatal binding index for ^{123}I-ILIS at 2 hr, expressed as the BI, was significantly higher in Rett patients (0.45 ± 0.04, mean ± s.e.m.) than in controls (0.23 ± 0.05, mean ± s.e.m.) ($p < 0.01$, unpaired t-test). No statistically significant difference was found between the two groups for the mean rCBF values nor for CBF estimated using the ILIS injected dose. No correlation was found between BI and striatal rCBF (Fig. 3). The BI at 2 hr could also not be correlated to age since the youngest children exhibited the highest as well as lowest values in both populations (Fig. 4). Among Rett patients no difference was found for BI either between epileptics and non-epileptics or between those with and without microcephaly. When rCBF values in the hemislice were normalized to the ILIS injected dose, no difference was found between

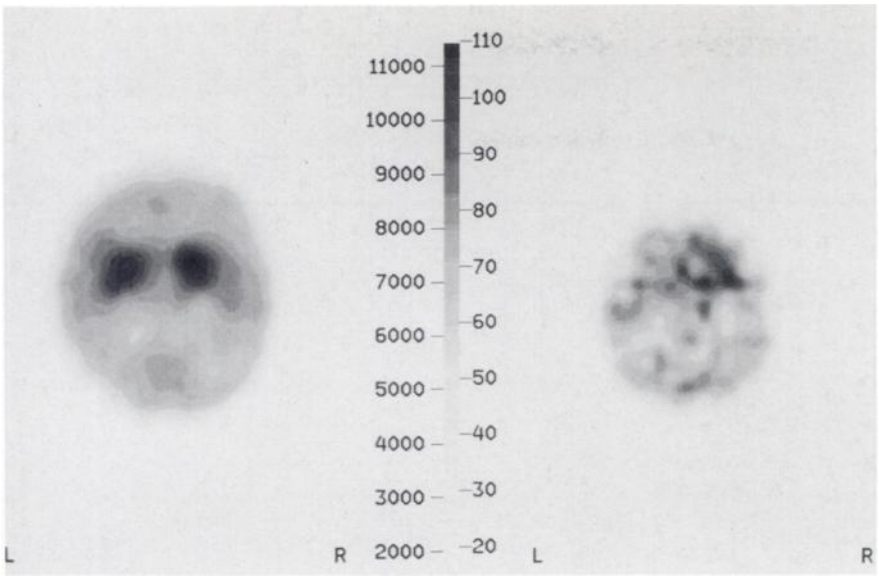

FIGURE 2. (Left) SPECT image of ^{123}I-ILIS binding obtained 2 hr after injection. (Right) Corresponding rCBF image obtained in the same patient and at the same level after injection of ^{133}Xe. The slice is 20 mm thick and includes both striata. The left scale represents radioactivity concentration; the right scale rCBF in ml/min/100 g.

Rett and control patients. All SPECT data are provided in Table 2.

DISCUSSION

The study shows a raised binding potential of ^{123}I-ILIS in the striatum of untreated children suffering from Rett syndrome. These results are consistent with an increased number (density) of striatal D2 receptors at the early stage of disease, as a result of reduced dopaminergic neurotransmission. Such an increase produces a rare condition which has only been seen in untreated Parkinson patients (*2*) and in schizophrenia (*15*), but the results are controversial (*16*).

Several methodological problems must be discussed. Iodolisuride has lower affinity for D2 receptors than other PET D2 ligands like butyrophenones, raclopride and bromolisuride (*17–19*). However, it has demonstrated usefulness for clinical studies (*20,21*) and could not miss as significant a change as a twofold increase of binding potential.

The nonspecifically bound fraction of the ligand is generally measured in cerebellum for D2 receptor studies (*17,22*). Using the SPECT camera, the cerebellar area was contaminated by very high radioactivity concentrations present in various infracerebral structures. Consequently, we decided to use the hemislice at the same level as the striatum as a reference area.

Microcephaly and cortical atrophy may induce changes in gamma-ray attenuation and in partial volume effect. Microcephaly may increase the binding index by decreasing the attenuation of gamma-rays emitted by internal structures. However, no relationship was found between the binding index and the existence of microcephaly. To reduce changes in the partial volume effect due to atrophy, which could affect the measurements performed in cortical structures, we selected the largest ROI possible, the whole hemislice, as the reference region.

Antiepileptic drugs, including the barbiturate we used as a premedication, are known to decrease rCBF (*23*). Whether or not they affect D2 binding is unknown. Since they were similarly administrated in Rett patients and in controls, they are unlikely to be the cause of the increase in binding observed in Rett patients.

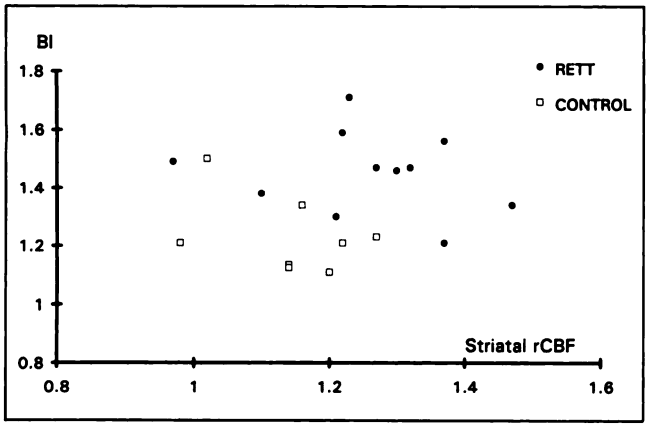

FIGURE 3. No linear correlation can be found between BI at 2 hr and striatal rCBF in this BI versus rCBF.

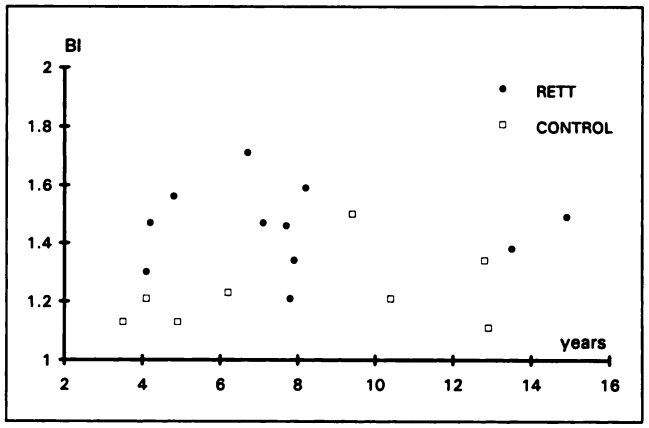

FIGURE 4. No linear correlation could be found between BI at 2 hr and age in this patient's SPECT-ILIS data.

TABLE 2
SPECT Data

Patients	L. BI*	R. BI*	Mean L-R BI*	L. rCBF**	R. rCBF**	Mean L-R rCBF**	Mean L-R HEM rCBF ILIS dose
Rett							
1	0.31	0.29	0.30	1.22	1.19	1.21	22.8
2	0.49	0.45	0.47	1.35	1.30	1.32	15.1
3	0.51	0.61	0.56	1.19	1.54	1.37	15.3
4	0.83	0.59	0.71	1.18	1.27	1.23	11.6
5	0.53	0.40	0.47	1.25	1.28	1.27	7.1
6	0.40	0.52	0.46	1.24	1.36	1.30	10
7	0.26	0.17	0.21	1.20	1.54	1.37	17.1
8	0.41	0.27	0.34	1.46	1.48	1.47	16.6
9	0.52	0.65	0.59	1.18	1.26	1.22	11.1
10	0.37	0.38	0.38	1.11	1.09	1.10	10.7
11	0.40	0.59	0.49	0.96	0.97	0.97	6.5
mean (s.e.m.)	0.46 (0.05)	0.45 (0.05)	0.45 (0.04)	1.21 (0.04)	1.30 (0.05)	1.26 (0.04)	13.1 (1.5)
Controls							
12	0.12	0.13	0.13	1.25	1.02	1.14	32.7
13	0.18	0.24	0.21	1.38	1.05	1.22	13.2
14	0.14	0.12	0.13	1.20	1.07	1.14	12.5
15	0.25	0.22	0.23	1.17	1.36	1.27	11.2
16	0.55	0.44	0.50	0.96	1.08	1.02	8.7
17	0.23	0.19	0.21	0.92	1.04	0.98	8.1
18	0.35	0.33	0.34	1.23	1.09	1.16	5.3
19	0.17	0.05	0.11	1.23	1.17	1.20	6.3
mean (s.e.m.)	0.25 (0.05)	0.22 (0.04)	0.23 (0.05)	1.17 (0.05)	1.11 (0.04)	1.14 (0.03)	12.3 (3.1)

*BI defined as striatum minus hemislice-to-hemislice radioactive equilibrium concentration ratio. rCBF expressed as rCBF/hemislice CBF; L = left; R = right; BI = Binding index; HEM = hemislice.

The control group is questionable. One of the main difficulties in pediatric functional brain studies performed with PET and SPECT is the collection of control values because normal children cannot be examined for ethical reasons. However, the need for normal values is particularly acute at this age because most functional parameters show dramatic changes during childhood due to the physiological postnatal maturation of the brain. D2-receptor density follows the same pattern as that described for glucose metabolism and rCBF (*12,24*): density increases during the first years of childhood and then decreases slowly to adult values, with no differences related to gender (*25*).

In the present work, patients selected as controls were paired for age. As in the Rett's group, the control group is a heterogeneous group with respect to epilepsy, a condition in which biogenic amines alterations have been reported (*26*). In the present series no difference in ^{123}I-ILIS binding was found between epileptic and nonepileptic patients. Our control group may therefore be considered reasonably representative of a normal population regarding striatal D2-receptor binding.

An increased binding potential due to a rCBF increase in the same area is presently excluded since striatal rCBF was simultaneously measured in every patient and did not differ in Rett patients or controls. Moreover, no relationship was found between binding index and rCBF. The two-fold increase in binding potential is then consistent with a doubling of D2-receptor density as compensation for dopamine deficiency. We make the hypothesis that the discordance of this result with the literature (*9*) may be due to the age of our patients. Reduced levels of dopamine are present very early in the disease (*27*) and could induce an "up-regulation" phenomenon detectable in the first years of life. However, an active degenerative process involves the dopaminergic nigrostriatal system and the basal forebrain cholinergic system (*5,28*). As a result, cell loss and morphological changes are observed later on in striatum, which could induce a decreased number of postsynaptic receptors in older patients.

Another explanation based on the possible competition between endogeneous and exogeneous ligands for receptor binding may also be proposed (*29,30*). The phenomenon varies according to the affinity of the ligands (*29*). A competition between endogeneous dopamine and ^{123}I-ILIS has recently been demonstrated in rats, using pharmacological manipulation of tissue dopamine concentrations (unpublished data). The decreased occupancy of D2 receptors by a low level of endogeneous dopamine would increase the density of free receptors and therefore result in an increased specific-binding potential. According to this hypothesis, discordant results with the literature could be due to the heterogeneity of Rett's disease. Clinical condition worsens with age but severity varies from one patient to another (*31*). Dopamine concentration in the central ner-

vous system was found in the normal range in some cases (*32*). Other neurotransmittors such as opioids are suspected to be involved (*33*). Dopaminergic impairment might therefore affect different patients at different levels, and the binding potential of D2 ligands might be variously altered from case to case. This could explain the normal values we found in a few patients and the absence of relationship between binding and age. SPECT D2 ligands with higher affinity than ^{123}I-ILIS could therefore have a better diagnostic value for atypical forms of Rett syndrome.

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