Short report

Failure of a peripheral dopaminergic marker in Parkinson's disease

J M MALOTEAUX, C E LATERRE, L HENS, P M LADURON

From the Department of Neurology, Université Catholique de Louvain Brussels, Department of Neurology, University of Leuven (K.U.L.), Leuven and Department of Biochemical Pharmacology, Janssen Pharmaceutica, Beerse, Belgium

SUMMARY [^3H]-spiperone binding on human lymphocytes did not reveal the occurrence of dopamine receptors. However, lower values were observed in Parkinsonism and the displaceable binding was increased after levodopa treatment although this was not specific only for levodopa and, furthermore, was not correlated with the clinical symptomatology. This non-specific binding in lymphocytes corresponds to trapping, presumably in lysosomes and thus does not reflect the dopaminergic receptors state in Parkinson's disease.

Degenerative processes or vascular disorder can, in the beginning, present a clinical picture very similar to idiopathic Parkinsonism but, in most cases, the diagnosis is not made different by the absence of a biological test for this disease. However, a biological marker could be very useful for studying the evolution of the disease, and for understanding fluctuations in response or progressive loss of efficacy of levodopa treatment. One recent approach of the dopaminergic system has been the intensive study of dopaminergic receptors performed with high affinity ligands such as [^3H]-spiperone¹ or [^3H]-haloperidol^{2,3} which can be used for *in vitro* binding studies with brain membranes or any other tissues.^{4,5} In man, [^3H]-spiperone binding to lymphocytes was reported as an index for the presence of dopaminergic receptors on these blood cells.⁶ As the number of binding sites was decreased in Parkinsonism, but became normal after levodopa treatment,⁷ this might be seen as an interesting biological parameter for the disease and as further evidence for a more generalised alteration of the dopaminergic system.⁸ Recently, we and other groups⁹⁻¹¹ demonstrated that the characteristics of [^3H]-spiperone binding to human lymphocytes were not similar to those of the striatal dopamine receptor (D2 site);

indeed there was no correlation between the IC_{50} values of dopamine antagonists in lymphocytes and in striatum and only a very weak stereospecificity was observed. Domperidone and sulpiride were found much less active in displacing [^3H]-spiperone than chloroquine, which does not possess anti-dopaminergic properties,¹⁰ but which is a known lysosomotropic agent.¹² We concluded that the displaceable [^3H]-spiperone binding to human lymphocytes corresponded to a trapping of the labelled ligand in the lymphocytes, presumably in the lysosomes. We now report on the [^3H]-spiperone binding in lymphocytes of patients treated or not with levodopa. Moreover the effects of levodopa and other compounds were also tested in *in vitro* binding with lymphocytes.

Patients and methods

[^3H]-Spiperone binding was performed on lymphocytes of a control group consisting of non-medicated healthy volunteers (23-75 years), and a second group consisting of Parkinsonian patients (57-79 years) who had never been treated before with levodopa and whose symptomatology was essentially akinetic. The same patients were seen 8 days after starting levodopa treatment. A further group consisted of Parkinsonian patients (59-70 years) who had had levodopa therapy at least for one year. The last group involved hospitalised patients (42-67 years) with neurological diseases but without extra-pyramidal symptoms (peripheral neuropathies, headache, etc). We excluded from this study patients treated with medications such as neuroleptics and antiemetics which are known to act on the

Address for reprint requests: Dr JM Maloteaux, Department of Neurology 1350, University of Louvain, 10, avenue Hippocrate, B-1200 Brussels, Belgium.

Received 19 March 1983 and in revised form 4 June 1983.
Accepted 28 June 1983

Fig Effect of various compounds on [³H]-spiperone binding in intact lymphocytes. Cells were preincubated for 90 min at 37°C and then assayed for [³H]-spiperone binding. "Specific binding" was defined as the difference between total spiperone binding and the binding in presence of 10⁻⁶ M haloperidol. Results are the mean value of experiments performed with blood samples from three non-medicated healthy volunteers in triplicate.

dopaminergic system, and sedative drugs. Data were analysed using Student's *t* test for detection of significant changes within a group. Difference was considered significant at the *p* < 0.05 level.

Heparinised blood (40 ml) was obtained from normal subjects or hospitalised patients. Lymphocytes were isolated on Ficoll Paque gradient. After hypotonic lysis of erythrocytes, the lymphocytes were counted in Coulter counter. Binding experiments were performed using 10⁶ cells and 5 nM [³H]-spiperone (specific activity 28 Ci/mmol, NEN, Boston). The displaceable binding ("specific") was defined as the difference between total binding and blank (in presence of 1 μM haloperidol). Binding assays were used as described elsewhere.^{1,6} For the *in vitro* binding assays with various drugs (fig), intact lymphocytes were incubated for 90 min at 37°C with the drug and then further incubated with [³H]-spiperone (5 nM) with or without 1 μM haloperidol.

Results

The table shows that, in each group of patients there was great variability for the values of "specific" [³H]-spiperone binding in lymphocytes. In spite of these variations, Parkinsonian patients seemed to have a lower binding than other patients or control subjects although interpretation of these results

Table Displaceable [³H]-spiperone binding in various patients

	fmol/10 ⁶ lymphocytes	n.
I. Controls	93 ± 38.1	17
II. Parkinsonian		
A. before treatment	27.0 ± 22.1	12*
B. 8 days after levodopa	59.6 ± 34.7	12*
C. 1 year levodopa treatment	84.2 ± 23.9	8
III. Miscellaneous nervous diseases	87.3 ± 20.5	10

Results are mean ± SD of binding experiments performed in triplicate.

n = number of patients.

**p* < 0.05 (between II.A and II.B and between I and II.A).

should thus be made with caution. The younger patients of the control group had slightly higher values of [³H]-spiperone binding than the five subjects who were more than 58 years. There was no correlation between the binding and the symptomatology of Parkinsonian patients and no difference was observed in binding parameters when on-off phenomenon occurred or when acute dystonia and dyskinesia were observed two hours after the intake of levodopa. All the binding values were significantly higher 8 days after treatment with levodopa in patients who did not receive this drug before (table). Two other patients without idiopathic Parkinson's disease but with motor impairment and vascular deficiency were treated for some weeks with levodopa and also had higher values 8 days after treatment (not quoted here). However it is not likely that such a change is specific only for levodopa. *In vitro* studies were performed to test the influence of levodopa, other amino acids and drugs on [³H]-spiperone binding in lymphocytes. As shown in the figure the displaceable binding was increased with several compounds such as levodopa, L-tyrosine, L-methionine but also acetylsalicylic acid and valproate. Therefore we were able to reproduce *in vitro* the results obtained *in vivo* with levodopa therapy but this *in vitro* effect is not restricted to levodopa alone. It should be noted that chloroquine, alprenolol or the opiate derivative R5573 at 100, 10 or 1 μM respectively inhibited [³H]-spiperone binding.

Discussion

Why lower values were observed in Parkinsonism remains unclear. Several parameters could strongly influence the displaceable binding such as the age of subjects, different lymphocyte subpopulations, cell fragility, membrane constitution and permeability or intracellular lysosomal content. The increase in the displaceable binding observed during levodopa treatment also appeared in non-Parkinsonian patients and was not restricted to levodopa in *in vitro* binding assays. Our observations might be explained by changes in the trapping properties of the lymphocytes. In accordance with the law of denervation, some postmortem studies showed that the level of dopamine receptors (D₂) was increased in the striatum of Parkinsonian patients¹³ and decreased with levodopa therapy. It was somewhat surprising that apparently opposite results were found in lymphocytes of such patients, a fact which strengthens the idea that [³H]-spiperone does not label dopamine receptors on lymphocytes. The [³H]-spiperone displaceable binding in lymphocytes must be regarded as a trapping phenomenon which

needs the intact cell because no displaceable binding occurred on membrane preparations of the cells. Trapping of labelled ligand was not restricted to lymphocytes but also occurred in cell cultures of neuronal origin.¹⁴ Morphological lysosomal changes have been described when neuronal cells in culture are exposed to chlorpromazine.¹⁵ At present it is not clear whether the trapping of neuroleptics or any other drugs with weak base properties may have some implications in the side effects of drugs. One may propose the hypothesis that the trapping into lysosomes of neuronal cells could be partly responsible for some long-term effects of neuroleptic drugs, as for instance tardive dyskinesia, where it is likely that a more general and more neurotoxic process than that resulting from the binding on specific dopamine receptors could be partially involved.

We thank C Waterkeyn for skilful technical assistance throughout this work. A part of this work was supported by IWONL. Dr JM Maloteaux is Aspirant of the Belgian FNRS.

References

- ¹ Leysen JE, Gommeren W, Laduron PM. Spiperone: a ligand of choice for neuroleptic receptors. 1. Kinetics and characteristics of *in vitro* binding. *Biochem Pharmacol* 1978;**27**:307-16.
- ² Seeman P, Chau-Wong M, Tedesco J, Wong K. Brain receptors for antipsychotic drugs and dopamine: direct binding assays. *Proc Natl Acad Sci USA* 1975;**72**:4376-80.
- ³ Creese I, Burt DR, Snyder SH. Dopamine receptor binding: differentiation of agonist and antagonist states with [³H]-dopamine and [³H]-haloperidol. *Life Sci* 1975;**17**:993-1002.
- ⁴ Brodie OE. Demonstration of vascular dopamine receptors in membrane from rabbit renal artery using [³H]-spiroperidol binding. *Experientia* 1981;**37**:1099-1101.
- ⁵ Magistretti PJ, Schorderet M. Dopamine receptors in bovine retina: characterization of the [³H]-spiroperidol binding and its use for screening dopamine receptor affinity of drugs. *Life Sci* 1979;**25**:1675-86.
- ⁶ Le Fur G, Meininger V, Phan T, Gerard D, Baulac M, Uzan A. Decrease in lymphocytes [³H]-spiroperidol binding sites in Parkinsonism. *Life Sci* 1980;**27**:1587-91.
- ⁷ Le Fur G, Meininger V, Baulac M, Phan T, Uzan A. Récepteurs dopaminergiques lymphocytaires et maladie de Parkinson idiopathique. *Rev Neurol (Paris)* 1981;**137**:89-96.
- ⁸ Larsen TA, Calne DB. Recent advances in the study of Parkinson's disease. *Trends Neurol Sci* 1982;**5**:10-12.
- ⁹ Bloxham CA, Cross AJ, Crow TJ, Owen F. Characteristics of [³H]-spiperone binding to human lymphocytes. *Br J Pharmacol* 1981;**74**:233P.
- ¹⁰ Maloteaux JM, Waterkeyn C, Laduron PM. Absence of dopamine and muscarinic receptors on human lymphocytes. *Arch Int Pharmacodyn Ther* 1982;**258**:174-6.
- ¹¹ Fleminger S, Jenner F, Marsden CD. Are dopamine receptors present on human lymphocytes? *J Pharm Pharmacol* 1982;**34**:658-63.
- ¹² de Duve C, de Barsey T, Poole B, Trouet A, Tulkens P, Van Hoof F. Lysosomotropic agents. *Biochem Pharmacol* 1974;**23**:2495-531.
- ¹³ Lee T, Seeman P, Rajput A, Farley IJ, Hornykiewicz O. Receptor bases for dopaminergic hypersensitivity in Parkinson's disease. *Nature* 1978;**273**:59-60.
- ¹⁴ Maloteaux JM, Gossuin A, Waterkeyn C, Laduron PM. Trapping of labelled ligands in intact cells: a pitfall in binding studies. *Biochem Pharmacol* 1983; in press.
- ¹⁵ Brosnan CF, Bunge MB, Murray M. The response of lysosomes in cultured neurons to chlorpromazine. *J Neuropath Exp Neurol* 1970;**29**:337-53.