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^{3}H-Spiroperidol Binding to Peripheral Mononuclear Cells in Schizophrenic and Healthy Subjects

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^{3}H-spiroperidol binding to peripheral blood mononuclear cells was measured in 28 patients, who fulfilled DSM-III-R-criteria for schizophrenia and 17 healthy subjects. There were no significant differences in characteristic binding parameters (K_d, B_{max}) between schizophrenic and healthy subjects. Moreover, there was no relation of binding parameters to any of the subtypes of schizophrenia or to the course of illness according to DSM-III-R-criteria. However, some patients exhibited higher B_{max} values without having a unique clinical symptomatology according to known diagnostic criteria. Neuroleptic treatment had no consistent effect on binding parameters intraindividually. K_d and B_{max} values were not related to age or gender. In conclusion, despite our previously reported improved methodology, we were not able to corroborate the clinical importance of this "peripheral marker" as a tool for diagnosing schizophrenia or for predicting the response to neuroleptic treatment in our sample of schizophrenic patients.

Key Words: Spiroperidol, lymphocytes, schizophrenia, peripheral model, neuroleptics

Introduction

In recent years research in the field of psychiatry focused on the evaluation of biological markers. Peripheral blood mononuclear cells (PBMC) are known to possess specific binding sites for several neurotransmitters, such as β-adrenergic receptors (Williams et al 1976), muscarinic cholinergic receptors (Strom et al 1974), receptors for substance P (Payan et al 1983), and vasoactive intestinal peptide (VIP) (Danek et al 1983).

As reported by LeFur et al (1980), murine and human PBMC reveal specific binding of the D_2-antagonist spiroperidol. These authors proposed this binding as a peripheral model of the central D_2-dopamine receptor site. Consequently, in line with the dopamine hypothesis, a significant and selective increase in binding capacity (B_{max}) for ^{3}H-spiroperidol in lymphocytes from schizophrenic patients was described (LeFur et al 1983; Rotstein et al 1983; Bondy et al 1984; Halbach and Henning 1989; Grodzicki et al 1990). Moreover, Bondy and Ackenheil (1987) reported a similar increase of B_{max} in healthy relatives of schizophrenics. This group suggested increased ^{3}H-spiroperidol binding as a genetic vulnerability marker for schizophrenia.

Interestingly, a decreased binding capacity has been detected in patients with idiopathic Parkinson's disease (LeFur et al 1980; Czlonkowska et al 1987; Bondy et al 1989). However, selection criteria of schizophrenic patients, methodological details, and even B_{max} and K_d values of healthy subjects differ widely between research groups. Moreover, considerable effort in various laboratories to reproduce these results failed (Bloxham et al 1981; Fle-

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minger et al 1982; Maloteaux et al 1982; Shaskan et al 1983; Feenstra et al 1989; Itzchaky et al 1989; Rao et al 1990). In previous reports we described in detail that some well-known, but obviously uncontrolled pitfalls in binding assays using viable cells might have contributed to this controversy. Moreover, because of an atypically shaped saturation curve, different mathematical methods to analyze the data were used throughout the literature (Wodarz et al 1989, 1992).

The potential clinical importance of ^{3}H-spiroperidol binding to lymphocytes as a tool to diagnose schizophrenia, to identify individuals at risk (Bondy and Ackenheil 1987) and/or to predict the response to neuroleptic treatment (Grodzicki et al 1990) led us to reinvestigate 28 patients fulfilling DSM-III-R-criteria of schizophrenia with our previously reported methodology.

Methods

Subjects

Venous blood was collected from 28 schizophrenic inpatients (21 men, 7 women; mean age 33.1 ± 12.4 [±SD] years) and 17 healthy individuals (14 men, 3 women; mean age 28.5 ± 11.9 years) in the morning hours between 8 and 10 AM. Patients fulfilled DSM-III-R-criteria for schizophrenia. DSM-III-R (APA 1987) diagnoses were made independently by two psychiatrists, who were blind to laboratory investigations. Summarized clinical and demographic data of the patients are given in Table 1.

Nine patients were free of oral drugs for at least 4 weeks and free of depot neuroleptics for at least 12 weeks at the time of laboratory testing. Three patients had never received neuroleptics before. The others received neuroleptics (haloperidol: 10–20 mg; clozapine: 150–500 mg per day) and some additional benzodiazepines (diazepam: 10–20 mg; lorazepam: 1–3 mg per day).

Five initially drug-free patients were reinvestigated after clinical improvement on maintenance therapeutic doses of haloperidol (decanoate 100–150 mg, every 4 weeks) or clozapine (200 mg; 250 mg daily). Healthy subjects and age-matched and gender-matched schizophrenic patients were studied in parallel on the same day. All subjects were carefully screened for nutritional status and prevalence of medical illness, as well as alcohol or nicotine abuse by medical history, physical examination and routine laboratory investigation. One woman and her matched control were postmenopausal, the others were tested during the midluteal phase of the cycle. Controls had no present or past evidence of a major psychiatric disorder according to DSM-III-R criteria and no family history of mental illness. They were free of any medication.

Informed consent was obtained from all subjects prior to the investigation. This study was approved by the ethical committee of the University of Würzburg and conformed to the declaration of Helsinki (last revision in Hong Kong, 1990).

Cell Preparation

Peripheral blood mononuclear cells (PBMC) were prepared immediately after venipuncture from ethylenediamine tetraacetic acid (EDTA) blood by sodium metrizoate-Ficoll density gradient centrifugation as described elsewhere in detail (Wodarz et al 1991). Briefly, blood was diluted with Hanks Balanced Salt Solution (HBSS) without Ca^{2+} and Mg^{2+} 1/1 (v/v) and layered carefully on sodium metrizoate-Ficoll. After centrifugation for 30 min (300 *g;* 23°C) the lymphocytes were obtained from the interphase. Lymphocytes were washed three times in Hanks Balanced Salt Solution without Ca^{2+} and Mg^{2+} (1/10 (v/v); 100 *g* for 10 min). The final cell count was performed with a Coulter Counter (Model S5, Coulter Electronics Ltd, GB). Viability of cells always exceeded 98%, as judged from their ability to exclude trypan blue. Routine staining was performed to assure a constant composition of cell suspensions.

Binding Assays

^{3}H-spiroperidol binding assays were performed immediately after cell separation using viable cells as described previously (Wodarz et al 1992). Cells were suspended in HEPES (25 mmol/L) buffered HBSS, pH 7.4 including 1.3 mmol/L Ca^{2+} and Mg^{2+}. To determine binding parameters, cells were incubated with 10 different concentrations of ^{3}H-spiroperidol (30 pM–3 nM) in the presence or absence of 1 μmol/L (+)-butaclamol in 96-well microtiter plates (NUNC, Sweden). Equilibrium was achieved by incubation at 37°C for 60 min in a humidified atmosphere (5% Co_2; 95% air). Free ligand was separated from bound ligand by rapid filtration through polyethyleneimine (PEI; 0.3%)-pretreated Whatman GF/C filters (Whatman, Maidstone, GB) with a Titertek semiautomatic cell harvester (Flow Laboratories, Inc., Rockville, MD) followed by an 11-sec wash with ice-cold HBSS. The radioisotope was measured by a standard liquid scintillation count in a Beckman LS 5000 TD counter at an efficiency of about 54%. All assays were routinely performed in duplicate.

Chemicals

^{3}H-spiroperidol (specific activity: 85–95 Ci/mol/L) was obtained from Amersham (Braunschweig, FRG). Sodium metrizoate-Ficoll and polyethyleneimine were obtained from Sigma Chemicals Inc. (St. Louis, USA). Hanks Balanced Salt Solution was obtained from Biochrom KG (Berlin,

Table 1. Binding Parameters (B_{max}, K_D; arithmetic mean ± SEM), as Estimated by Three Different Modes of Calculation (see Methods for details)

	(a) one saturable site			
	Healthy subjects (n = 17)	Schizophrenic patients (n = 28)	t	p
K_D [nmol/L]:	3.21 ± 0.50	2.69 ± 0.25	1.03	0.3
B_{max} [fMol/10^6 cells]:	7.82 ± 1.30	8.88 ± 1.22	−0.57	0.6
	(b) one saturable site and omitting the nonsaturable part			
	Healthy subjects	Schizophrenic patients	t	p
K_D [nmol/L]:	0.31 ± 0.04	0.31 ± 0.04	0.09	0.9
B_{max} [fMol/10^6 cells]	1.12 ± 0.16	1.88 ± 0.41	−1.43	0.2
	(c) one saturable site and one nonspecific, nonsaturable part			
	Healthy subjects	Schizophrenic patients		
K_D [nmol/L]:	0.21 ± 0.04	0.18 ± 0.02	0.94	0.4
B_{max} [fMol/10^6 cells]	0.55 ± 0.08	0.62 ± 0.08	−0.51	0.6

Binding parameters K_d and B_{max} (mean ± SEM) of ^{3}H-spiroperidol binding to unfractionated lymphocytes: Calculation based on the assumptions: (a) one saturable site in the ligand concentration range up to 3 nmol/L; (b) one saturable site (the nonsaturable part in the ligand concentration range 0.03 nmol/L–3 nmol/L was omitted); (c) one saturable site and one nonspecific nonsaturable part within the ligand concentration range 0.03 nmol/L–3 nmol/L. There were no statistically significant differences between healthy and schizophrenic subjects, irrespective of the method of calculation.

FRG), HEPES from Serva Chemicals (Heidelberg, FRG), (+)-butaclamol from Research Biochemicals Inc. (Wayland, USA). All dilutions were made with HBSS. Because some subsets of PBMC are known to adhere to glassware, only plastic material was used throughout the experiments.

Data Analysis

The term "displaceable binding" defines the difference between binding in the absence and in the presence of 1 μmol/L (+)-butaclamol. K_d and B_{max} values were derived from computerized iterative nonlinear regression analysis (Enzfitter, Elsevier Biosoft). The Enzfitter-software offers the option to weigh the data statistically and exclude "outliers." The same results could be obtained by excluding samples with a coefficient of variation within a single experiment exceeding 10%.

The following equations were used to fit the binding curves: (a,b) single-site model: Bound = $(B_{max} \times \text{Free})/(K_d + \text{Free})$; (c) one specific and one nonspecific binding site model:

$$\text{Bound} = (B_{max} \times \text{Free})/(K_d + \text{Free}) + (F \times \text{Free})$$

where F represents the slope of the nonspecific but dispalceable binding. Data were analyzed using Pearson's product-moment correlation, t-test for independent or dependent variables where appropriate, and analysis of covariance (ANCOVA). All significance levels are two-tailed.

Results

The saturation experiments in healthy and schizophrenic subjects revealed a biphasic saturation-curve with an initial part at low ligand concentrations (<1 nmol/L) and a second linear part with higher ligand concentrations (1–300 nmol/L), which is not saturable. The mathematical methods described above for the calculation of B_{max} and K_d values were applied to this atypically shaped "saturation curve." The results are shown in Table 1.

The intraindividual coefficient of variation was below 10%. However, there was a large interindividual variation of B_{max} and K_d values in healthy and schizophrenic subjects. The amount of interindividual and intraindividual scattering depended on the mode of calculation (Table 1).

The same statistical results were obtained, irrespective of the mathematical model applied to analyze the "saturation curve." We could previously demonstrate that the complex model of one specific and one nonspecific binding site is the most adequate to describe this atypically shaped "saturation curve" (Wodarz et al 1992). Therefore, data are presented only for this model. There were no statistically significant differences in B_{max} and K_d values between the schizophrenic and healthy subjects. Moreover,

Fig. 1a

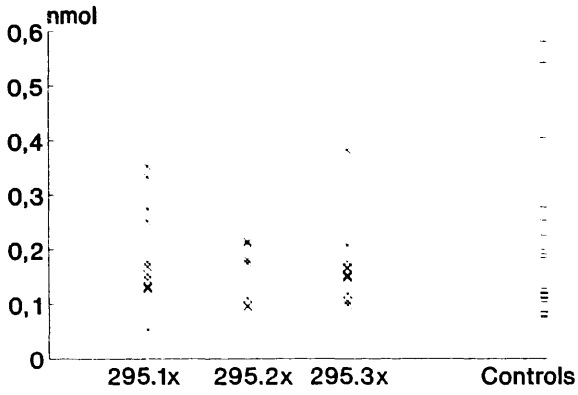

Fig. 1b

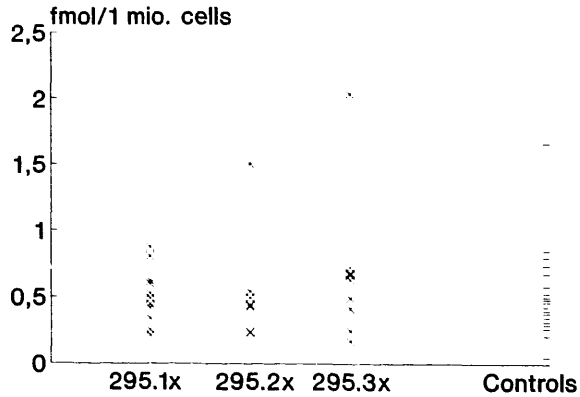

Figure 1. Binding affinity (K_d, Figure 1a) and binding capacity (B_{max}, Figure 1b) of ^{3}H-spiroperidol to PBMC of 17 healthy subjects (−) and 28 schizophrenic patients (x). Patients were subclassified to type of schizophrenia according to DSM-III-R-criteria (295.1x; 295.2x; 295.3x). The fifth digit code "x" describes that the course of the disease was ignored. There were no statistically significant differences in the characteristic binding parameters between the different subtypes of schizophrenic disorder or to the controls. Binding parameters K_d and B_{max} were calculated by using the model of one specific and one nonspecific binding site (Le Fur et al 1983; Bidart et al 1983; Wodarz et al 1992).

Fig. 2a

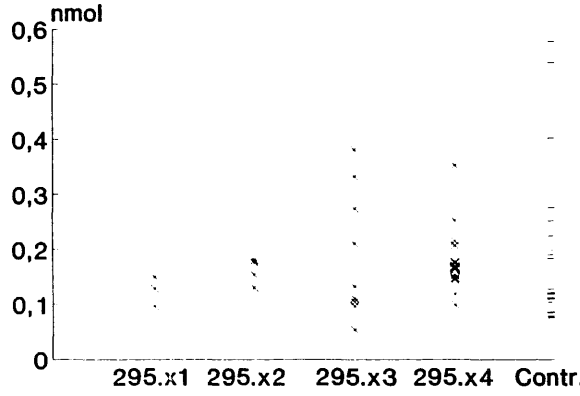

Fig. 2b

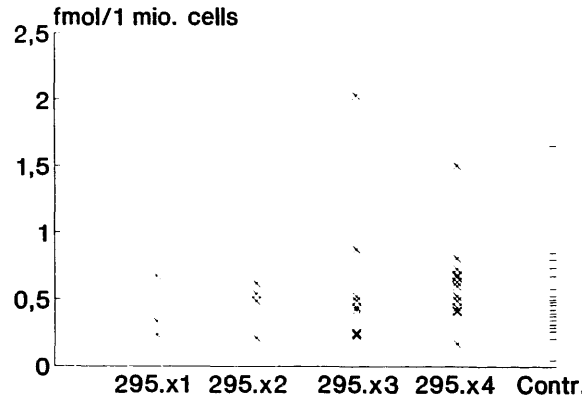

Figure 2. Binding affinity (K_d, Figure 2a) and binding capacity (B_{max}, Figure 2b) of ^{3}H-spiroperidol to PBMC of 17 healthy subjects (−) and 28 schizophrenic patients (x). Patients were subclassified to course of schizophrenic disorder according to DSM-III-R-criteria (295x1; 295x2; 295x3; 295x4). The fourth-digit code "x" describes that the subtype of the schizophrenic disorder was ignored. There were no statistically significant differences in the characteristic binding parameters between the different courses of the disease or to the controls. Binding parameters K_d and B_{max} were calculated according to the model of one specific and one nonspecific binding site (Le Fur et al 1983; Bidart et al 1983; Wodarz et al 1992).

there was no relation of characteristic binding parameters to any of the subtypes of schizophrenia or to the course of illness according to DSM-III-R-criteria (Figures 1 and 2).

Drug-free patients exhibited no significant differences in binding parameters compared to healthy subjects or pharmacologically treated patients. Moreover, there were no significant changes in intraindividual B_{max}, K_d and F-values in five initially drug-free patients, who were reinvestigated after clinical improvement on maintenance therapeutic doses of haloperidol or clozapine (Figure 3).

Nevertheless, there were some patients, whose binding parameters where apparently different from those of the other subjects (Figures 1 and 2). Those patients exhibited higher B_{max} values with all mathematical models applied to analyze the "saturation curve." However, we were unable to establish a unique clinical symptomatology or course of the disorder according to DSM-III-R-criteria. In addi-

Fig. 3a

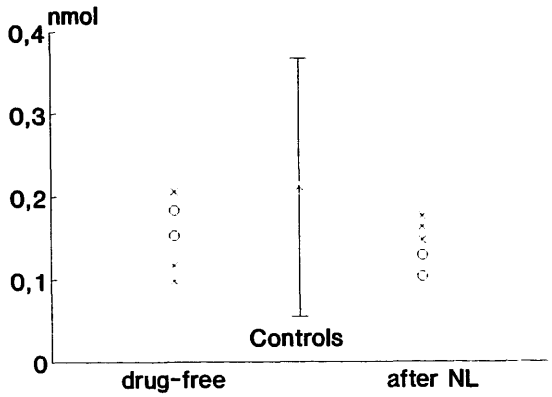

Fig. 3b

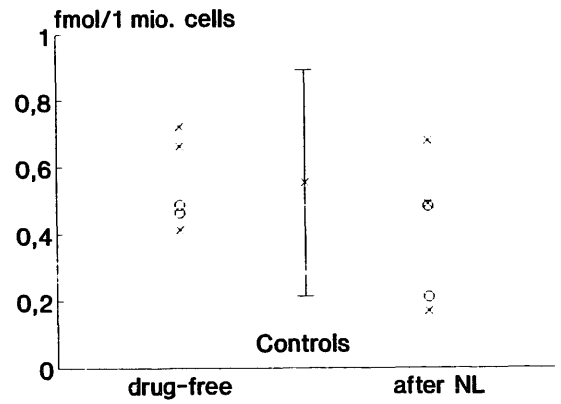

Figure 3. Binding affinity (K_d, Figure 3a) and binding capacity (B_{max}, Figure 3b) of ^{3}H-spiroperidol to PBMC of 5 drug-free schizophrenic patients, who were reinvestigated after treatment with haloperidol (x) or clozapine (o). Mean ± SD of control subjects is given as a reference.

tion, there was no significant influence of age or gender on the binding parameters using Pearson's product-moment correlation and ANCOVA.

Discussion

Initially, LeFur et al (1980b, 1983) reported an increase in ^{3}H-spiroperidol binding sites on peripheral lymphocytes in schizophrenic patients and a decrease of these binding sites in idiopathic Parkinson's disease. Although some groups could confirm these findings in schizophrenics (Rotstein et al 1983; Bondy et al 1984; Halbach and Henning 1989; Grodzicki et al 1990) and patients with Parkinson's disease (Czlonkowska et al 1987; Bondy et al 1989), others were not able to replicate a binding site for ^{3}H-spiroperidol in PBMC at all (Bloxham et al 1981; Fleminger et al 1982; Maloteaux et al 1982; Shaskan et al 1983; Feenstra et al 1989; Itzchaky et al 1989; Rao et al 1990). We previously described in detail some fundamental methodological issues, which might have contributed to this discrepancy (Wodarz et al 1992).

In previous studies paranoid schizophrenics (LeFur et al 1983; Rotstein et al 1983) were reported to exhibit the highest B_{max} values. In contrast, our schizophrenic patients with a paranoid subtype according to DSM-III-R-criteria did not show significantly different binding parameters compared to healthy individuals. Moreover, if the patients were subclassified to type or course of schizophrenic disorder according to DSM-III-R-criteria, there were no statistically significant differences in any of the characteristic binding parameters (B_{max}, K_d) between the subgroups or compared to healthy subjects. However, there were three patients out of 28, whose B_{max} values were more than one SD above the arithmetic mean. These three patients did not exhibit a unique clinical symptomatology or course of the disease according to DSM-III-R criteria. These patients might well belong to a subgroup of schizophrenic patients, whose disease might have a unique etiology and need a unique type of treatment. This might be supported by the actually preliminary observation that those patients did not respond well to neuroleptic treatment. However, some other patients were nonresponders to standard neuroleptics as well without having elevated B_{max} values. This is in contrast to a previous study (Grodzicki et al 1990), which observed a striking difference in binding parameters between neuroleptic responsive and nonresponsive patients.

Moreover, LeFur et al (1983) described a decrease in binding capacity B_{max} of drug-free patients after short-time neuroleptic treatment and an increase after long-time administration of neuroleptics. However, we could not observe this phenomenon in our five drug-free patients, who were on neuroleptic treatment at the time of reinvestigation. It did not matter whether the typical neuroleptic haloperidol or the atypical one clozapine was used.

As we previously have described in detail (Wodarz et al 1992), some well-known but obviously uncontrolled methodological issues might have contributed to this controversial results (e.g., unspecific filter binding, which increases in the presence of (+)-butaclamol as a displacer, or a variable amount of contaminating granulocytes, which do not exhibit the spiroperidol binding site). Moreover, selection criteria of schizophrenic patients differed between the groups. LeFur et al (1983) classified their patients in type I syndrome (acute schizophrenics with positive symptoms) and type II syndrome (chronic schizophrenics with negative symptoms). Bondy et al (1984) and Halbach and Henning (1989) classified their patients according to International Classification of Diseases (ICD)

9 criteria, but mentioned that only a minority of patients fulfilled DSM-III-R criteria of schizophrenia, as the majority of their patients suffered from their first and acute episode of illness. Rao et al (1990) described ICD-9 diagnoses, too. Rotstein et al (1983) used the Research Diagnostic Criteria. Only Itzchaky et al (1989) and Grodzicki et al (1990) classified their patients according to DSM-III-R criteria. Most of the patients in our study were chronically ill (duration of illness >1 year).

In conclusion, we were not able to corroborate the clinical importance of this "peripheral marker" as a tool for diagnosing schizophrenia or for a prediction of response to neuroleptic treatment in our sample of schizophrenic patients. Apart from not exactly knowing the pharmacological and molecular nature of the "^{3}H-spiroperidol binding site" in peripheral blood mononuclear cells, its physiological significance remains to be evaluated.

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