^{3}H-Spiroperidol Binding to Human Peripheral Mononuclear Cells: Methodological Aspects

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^{3}H-spiroperidol binding to lymphocytes has been proposed as a vulnerability marker for schizophrenia. However, the biological significance and even existence of this "binding site" are still in controversy. Therefore, the present study reevaluated methodological details using a filtration binding assay. The results indicated that some well-known, but obviously uncontrolled pitfalls might contribute to this controversy [e.g., unspecific filter binding, which increased in the presence of (+)-butaclamol, or a variable amount of contaminating granulocytes). Moreover, due to an atypically shaped saturation curve, different mathematical methods to analyze the data were used and compared. The present data should help us to understand the biological relevance of this marker, as viewed in different laboratories.

Introduction

In recent years research in the field of psychiatry focused on the evaluation of biological markers. For reasons of clarity it is of benefit to distinguish state-dependent (state) from state-independent (trait) markers. A state-independent feature could improve diagnostic validity and identify subgroups. Moreover, it might make it possible to identify individuals at risk. Recently, increased binding of ^{3}H-spiroperidol to peripheral lymphocytes was reported to be a trait marker in schizophrenic patients (Bondy and Ackenheil 1987).

The pathophysiology of schizophrenia is still not known. To date the dopamine hypothesis of schizophrenia is favored, since antipsychotic drugs are thought to act by blocking D_2 dopamine receptors (Seeman et al 1976). Previous evidence from postmortem studies that showed significantly increased striatal D_2 dopamine receptors in schizophrenic patients (Cross et al 1981; Mita et al 1986) has recently been ascribed to neuroleptic treatment prior to death (Kornhuber et al 1989). In vivo studies using positron emission tomography (PET) have demonstrated elevated D_2 dopamine receptors in schizophrenia (Crawley et al 1986; Wong et al 1986), but these findings could not be replicated in other studies (Farde et al 1988; Martinot et al 1990).

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LeFur et al (1980a) initially reported the presence of ^{3}H-spiroperidol binding sites on rat and human peripheral lymphocytes as a model of a peripheral D_2 dopamine receptor site. Consequently, in agreement with the dopamine hypothesis, a significant and selective increase in binding capacity (B_{max}) for ^{3}H-spiroperidol in lymphocytes of schizophrenic patients was described (LeFur et al 1983; Rotstein et al 1983; Bondy et al 1984; Halbach and Henning 1989; Grodzicki et al 1990). However, selection criteria of patients, methodological details and even B_{max} and K_d values of healthy subjects have differed widely between research groups.

A similar increase of B_{max} was reported in relatives of schizophrenics (Bondy and Ackenheil 1987) and suggests 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 1980b; Czlonkowska et al 1987; Bondy et al 1989).

In contrast, considerable effort in various laboratories to corroborate these results failed (Bloxham et al 1981; Fleminger et al 1982; Maloteaux et al 1982; Shaskan et al 1983; Feenstra et al 1989; Rao et al 1990). In view of the potential clinical relevance and the variety of pitfalls inherent in binding assays using viable cells, the present study reevaluated some methodological issues, including the mathematical procedures applied to analyze the data.

Methods

Subjects

Venous blood was collected from 12 healthy men [mean age 28.1 ± 13.1 (±SD) years] between 8:00 and 10:00 AM. Subjects with any present illness or under medication, alcohol or nicotine abusers, and persons with a history of stressful life events during the month prior to the investigation were excluded.

To control for constancy and reproducibility, binding parameters of lymphocytes from three subjects were measured in blood samples obtained at least on two separate days. The influence of storage on composition of cell suspensions was studied in three subjects where the EDTA-blood samples were kept for 1, 2, 3, 5, 8, 10, and 12 hr at room temperature.

Cell Preparation

Peripheral blood mononuclear cells (PBMC) were prepared immediately after venipuncture from 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 and the granulocytes from the pellet (hemolysis of erythrocytes with 0.8% ammonium chloride). Lymphocytes and granulocytes 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 (Pappenheimer 1917). Enriched B and T lymphocytes were obtained after culture of unfractionated PBMC for 10 days in culture

medium supplemented with 10 μg/ml pokeweed mitogen (PWM) or 10 μg/ml phytohemagglutinin A (PHA). Routine staining was performed to ensure a constant composition of cell suspensions.

Membrane Preparation

Crude lymphocyte membranes were prepared from one individual by repeated freeze thawing at −80°C. The preparation was homogenized with a Teflon homogenizer with six up-and-down strokes at 1000 rpm. The homogenate was washed three times in HBSS without Ca^{2+} and Mg^{2+} (20.000 *g* for 15 min) and used immediately afterwards for a saturation experiment. About 13 μg per well membrane protein was used. This corresponded to the amount of viable cells in the binding assays. Protein was determined according to Lowry et al (1951).

Binding Assays

^{3}H-spiroperidol binding assays were performed immediately after cell separation using viable cells (except where otherwise stated). 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 ten different concentrations of ^{3}H-spiroperidol (30 pmol/L to 3 nmol/L) 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). To determine saturability of binding, ^{3}H-spiroperidol concentrations up to 300 nmol/L were used. Free was separated from bound ligand by rapid filtration through polyethyleneimine (PEI; 0.3%; Sigma, St. Louis, USA)-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, except where otherwise stated.

Determination of Unspecific Filter Binding

To determine unspecific binding of ^{3}H-spiroperidol to the widely used Whatman GF/B and Whatman GF/C glass-fiber filters, a binding assay was performed as described above without addition of cells. Different methods were tested to reduce unspecific binding of radioligand to the glass-fiber filters (e.g., extended washing time, pretreatment of filters with different concentrations of the polycationic polyethylenimine).

Displacement of ^{3}H-Spiroperidol Binding

Various concentrations of (+)- and (−)-butaclamol (10^{-10}–10^{-2} mol/L) and GBR 12909 were used to displace ^{3}H-spiroperidol from its binding site.

Chemicals

^{3}H-spiroperidol (specific activity 85–96 Ci/mmol/L, except where indicated) was obtained from Amersham (Braunschweig, Germany). Ammonium chloride, sodium metrizoate-Ficoll, trypan blue, phytohemagglutinin A, and pokeweed mitogen were obtained from

Sigma Chemicals Inc. (St. Louis, USA). GBR 12909 was obtained from Research Biochemicals Inc. (Natick, USA).

Culture medium consisted of Rosewell Park Memorial Institute Medium (RPMI) 1640 (Boehringer, Mannheim, Germany) supplemented with 2 mmol/L L-glutamate, 0.1 mg/ml gentamycine, and 10% (v/v) fetal calf serum (all Biochrom KG, Berlin, Germany). Hanks Balanced Salt Solution was obtained from Biochrom KG (Berlin, Germany), HEPES from Serva Chemicals (Heidelberg, Germany), and (+)- and (−)-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 of weighing the data statistically and excluding "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 analyze the data and to fit the binding curves:

(a,b) single-site model:

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

(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 displaceable binding;

(d) two site model:

$$\text{Bound} = (B_{max1} \times \text{Free})/(K_{d1} + \text{Free}) + (B_{max2} \times \text{Free})/(K_{d2} + \text{Free}).$$

To determine whether the increase of goodness of fit for a model with additional parameters was significantly more than could be expected on the basis of chance alone, a reliable F test was applied:

$$F = (SS_1 - SS_2)/(df_1 - df_2)/(SS_2/df_2),$$

where SS_1 and SS_2 are the residual sums of squares of the deviations of the points to the fitted curve, and df_1 and df_2 are the associated degrees of freedom (numbers of data points minus number of parameters) for the original model and the model with additional parameters, respectively (Munson and Rodbard 1980; Bardsley and McGinlay 1987).

Results

Separation of Free from Bound ^{3}H-Spiroperidol

Using Whatman GF/B filters, 50%–80% of total binding was unspecific binding of radioligand to filter material (Figure 1). Only about 5%–15% corresponded to displaceable binding. Moreover, unspecific binding of ^{3}H-spiroperidol to glass-fiber filters increased

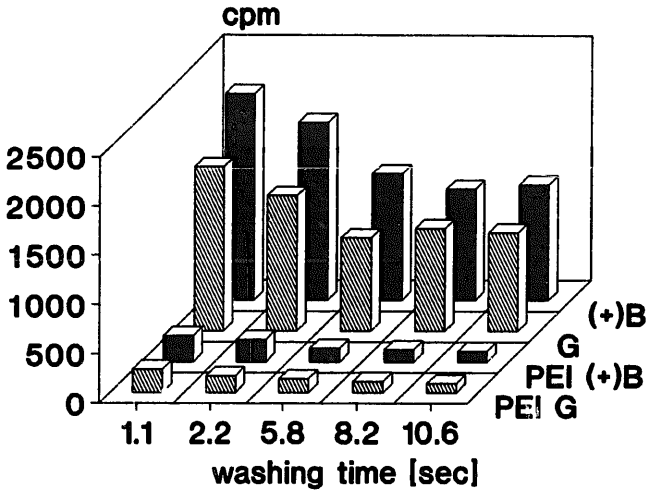

Figure 1. Counts-per-minute (cpm) values of unspecific ^{3}H-spiroperidol binding to Whatman GF/B filters with and without pretreatment with 0.3% polyethyleneimine [PEI]. Note that unspecific filter binding initially was higher in the presence of 1 μmol/L (+)-butaclamol [(+)-B] as displacing agent (without (+)-butaclamol [G]). Moreover, reduction of unspecific filter binding by extended washing was insufficient. Results are the mean of three different experiments with 3 nmol/L ^{3}H-spiroperidol (totals: 12,391 cpm); standard deviations were omitted for reasons of clarity.

to 65%–90% in the presence of (+)-butaclamol (Figure 1). Consequently, the calculated displaceable binding decreased substantially. Figure 1 demonstrates that extended washing halved unspecific filter binding. Pretreating Whatman GF/C filters (results with other filter material are not shown) with 0.3% polyethyleneimine (PEI) (Bruns et al 1983) reduced unspecific filter binding to 5%–10% of total binding. Moreover, displaceable binding increased to 40%–60% of total binding and the experimental intraassay and interassay scattering decreased. The coefficient of variance without PEI was 0.36; with PEI, it was 0.09.

Displacement of ^{3}H-Spiroperidol Binding

The enantiomers of butaclamol exhibited no stereoselectivity with high (> 2 nmol/L) concentrations of ^{3}H-spiroperidol (Figure 2a). However, by using low (< 1 nmol/L) concentrations of ^{3}H-spiroperidol (+)-butaclamol was about 8 times more potent than (−)-butaclamol (Figure 2b). GBR 12909, a selective dopamine uptake inhibitor (Heikkila and Manzino 1984), was about 100 times more potent than (−)-butaclamol in displacing low concentrations of ^{3}H-spiroperidol, but it was hardly effective with high (> 2 nmol/L) concentrations of radioligand.

^{3}H-Spiroperidol Binding to Subsets of Peripheral Blood Mononuclear Cells and Membrane Preparations

We investigated ^{3}H-spiroperidol binding to unfractionated lymphocytes, to enriched B and T lymphocyte-suspensions, and to granulocytes. The typically shaped saturation curve (Figure 3) could only be found with unfractionated lymphocytes and in enriched B cell fractions. T cells and granulocytes exhibited only nonspecific binding within the ligand concentration range used (30 pmol/L to 3 nmol/L).

There was a linear relationship between nonspecific binding and the amount of contaminating granulocytes, that is, the percentage of nonspecific binding increased as the number of granulocytes increased ($r = 0.88, p < 0.05$). Moreover, there was a negative

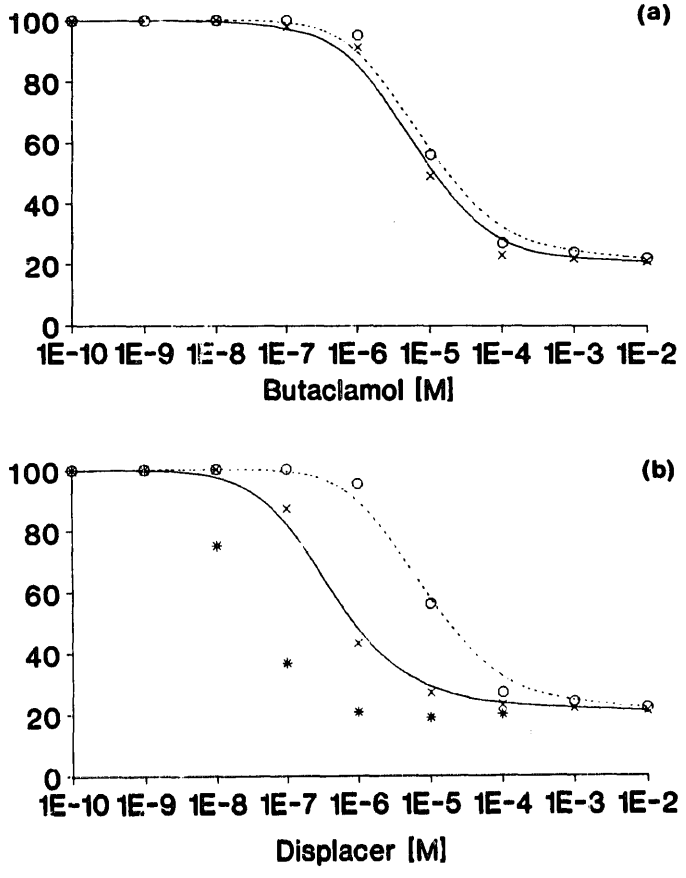

Figure 2. (a) x——x, (+)-butaclamol; o---o, (−)-butaclamol. Results are the mean of three different experiments, each carried out in triplicate. Displacement curves with 3 and 0.4 nmol/L were established simultaneously with the same PBMC suspensions. By using a ligand concentration of 3 nmol/L ^{3}H-spiroperidol displacement curves of (+)- and (−)-butaclamol revealed no stereoselectivity. (b) x——x, (+)-butaclamol; o---o, (−)-butaclamol; * . . . *, GBR 12909. Results are the mean of three different experiments, each carried out in triplicate. Displacement curves with 0.4 and 3 nmol/L were established simultaneously with the same PBMC suspensions. By using a ligand concentration of 0.4 nmol/L ^{3}H-spiroperidol displacement curves exhibited that (+)-butaclamol is about 8 times more potent than (−)-butaclamol. GBR 12909, a selective dopamine uptake inhibitor, was about 100 times more potent than (−)-butaclamol.

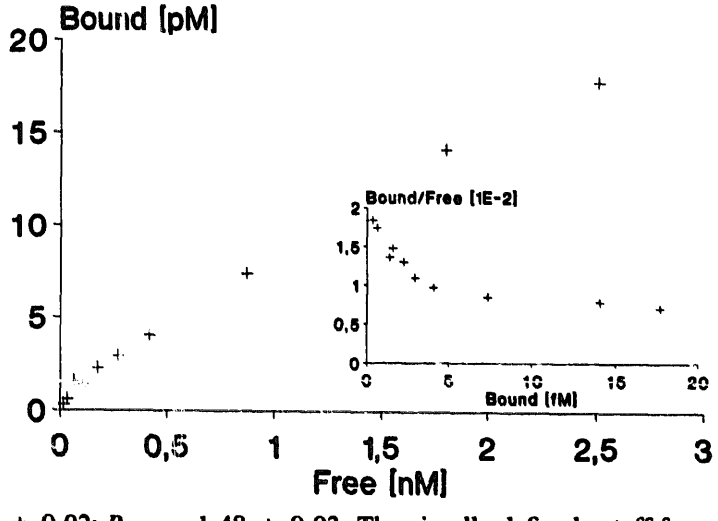

Figure 3. Typically atypical saturation curve of ^{3}H-spiroperidol (ligand concentration range: 0.03–3 nmol/L) to unfractionated lymphocytes. The inset displays the linear transformation by Scatchard (1949). Only a total of 2%–4% of free ligand was bound to the cells, of which 47%–62% was displaceable by 1 μmol/L (+)-butaclamol. (a) One saturable site: K_d = 3.18 ± 0.24; B_{max} = 7.94 ± 0.64. (b) One saturable site and omitting the nonsaturable part: K_d = 0.38 ± 0.02; B_{max} = 1.48 ± 0.03. The visually defined cutoff for omitting the nonsaturable part was above 0.5 nmol/L free ligand. (c) One saturable site and one nonspecific nonsaturable part: K_d = 0.122 ± 0.010; B_{max} = 0.360 ± 0.094. The curve fits were omitted for reasons of clarity.

Table 1. Binding Parameters (B_{max}, K_d), Dependent on Mode of Calculation[a]

	(a) One Saturable Site		
	Bidart et al (1983)	LeFur et al (1980a, 1980b)	Wodarz et al
K_d (nmol/L)	2.27 ± ?	5.4 ± 0.5	2.69 ± 0.41
B_{max} (fmol/10^6 cells)	29 ± ?	103 ± 4	6.15 ± 0.96
	(b) One Saturable Site and Omitting the Nonsaturable Part		
	Halbach and Henning (1989)	Grodzicki et al (1990)	Wodarz et al
K_d (nmol/L)	Not stated	0.18 ± 0.03	0.36 ± 0.04
B_{max} (fmol/10^6 cells)	1 ± 0.6	3.4 ± 1.2	1.18 ± 0.15
	(c) One Saturable Site and One Nonspecific Nonsaturable Part		
	Bondy and Ackenheil (1987)	Wodarz et al	
K_d (nmol/L)	0.17 ± 0.07	0.25 ± 0.05	
B_{max} (fmol/10^6 cells)	2.66 ± 0.69	0.50 ± 0.06	

[a]Calculation based on the following asumptions: (a) one saturable site in the ligand concentration range up to 5 nmol/L; (b) one saturable site (the nonsaturable part in the ligand concentration range 0.03–3 nmol/L was omitted); *Note*—Grodzicki et al used a ligand concentration range of 0.07–1.2 nmol/L; and (c) one saturable site and one nonspecific nonsaturable part within the ligand concentration range 0.03–3 nmol/L.

correlation between time of storage of EDTA-blood and amount of displaceable binding ($r = -0.85$, $p < 0.05$). Routine staining revealed that this was due to an increased contamination by granulocytes.

In crude membrane preparations we were unable to detect displaceable binding.

Saturation Experiments

Our experiments in healthy volunteers revealed a biphasic saturation curve with an initial part at low ligand concentrations (< 1 nmol/L), which might be interpreted as a saturable binding site and a second linear part with higher ligand concentrations (1–300 nmol/L), which is not saturable (Figure 3).

The mathematical methods described above for the calculation of B_{max} and K_d values were applied to this atypically shaped saturation curve (Table 1).

In 10 of 12 individuals tested, it was not possible to obtain B_{max} and K_d values of two saturable binding sites.

Because the goodness of fit will always improve when additional parameters are added to a model, we applied a reliable F test for model discrimination (Munson and Rodbard 1980; Bardsley and McGinlay 1987). This shows that the model of one specific and one nonspecific binding site significantly improves the goodness of fit, and is preferable to the simpler model of one single site in the ligand concentration range 0.03–3 nmol/L ^{3}H-spiperone ($F = 6.67$, $df_1 = 8$, $df_2 = 7$, $p < 0.05$). The single-site model, which omits

Table 2. Binding Parameters K_d and B_{max} (mean ± SD) of ^{3}H-Spiroperidol Binding to Unfractionated Lymphocytes in Three Individuals Tested on Different Days[a]

Subject	Day	(a) One Saturable Site K_d (nmol/L)	B_{max} (fmol/10^6 cells)
1	1	3.55 ± 0.24	9.83 ± 0.52
	2	2.56 ± 0.10	12.01 ± 0.75
2	1	1.99 ± 0.12	5.81 ± 0.37
	2	2.81 ± 0.31	6.69 ± 0.56
3	1	5.63 ± 0.30	5.88 ± 0.95
	2	4.00 ± 0.57	6.97 ± 0.84
	3	3.17 ± 0.21	4.09 ± 0.55
Subject	Day	(b) One Saturable Site and Omitting the Nonsaturable Part K_d (nmol/L)	B_{max} (fmol/10^6 cells)
1	1	0.454 ± 0.022	2.261 ± 0.052
	2	0.449 ± 0.014	2.469 ± 0.375
2	1	0.191 ± 0.019	5.313 ± 0.037
	2	0.227 ± 0.031	5.026 ± 0.145
3	1	0.388 ± 0.021	7.390 ± 0.195
	2	0.295 ± 0.013	6.209 ± 0.184
	3	0.179 ± 0.021	8.091 ± 0.515
Subject	Day	(c) One Saturable Site and One Nonspecific, Nonsaturable Part K_d (nmol/L)	B_{max} (fmol/10^6 cells)
1	1	0.121 ± 0.013	0.792 ± 0.064
	2	0.134 ± 0.015	0.928 ± 0.110
2	1	0.165 ± 0.019	0.608 ± 0.032
	2	0.174 ± 0.018	0.719 ± 0.077
3	1	0.198 ± 0.017	0.238 ± 0.030
	2	0.183 ± 0.011	0.185 ± 0.016
	3	0.179 ± 0.021	0.312 ± 0.035

[a]The calcuations are based on the assumptions described in the text. The individual SD is given by the software used for nonlinear regression data analysis to indicate the goodness of fit.

the nonsaturable part (ligand concentration range 0.05–3 nmol/L) (Halbach and Henning 1989), significantly improves the goodness of fit too ($F = 33.6$, $df_1 = 8$, $df_2 = 3$, $p < 0.005$). However, this model is not adequate, as discussed later.

The K_d and B_{max} values exhibited a considerable variation between individuals, but remained relatively constant in repeated measurements intraindividually. The amount of interindividual and intraindividual scattering depends on the mode of calculation (see Table 2 and SD in Table 1).

Discussion

In line with the dopamine hypothesis, LeFur et al (1980a, 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. Subsequently, these exciting results were questioned, because even the methodology applied could not be constantly reproduced in other laboratories. The potential clinical importance of ^{3}H-

spiroperidol binding to lymphocytes as a "vulnerability marker of schizophrenia" (Bondy and Ackenheil 1987) led us to reinvestigate the most striking pitfalls in binding assays using viable cells.

Separating free from bound ligand is a critical point in determination of the characteristic binding parameters B_{max} and K_d. Due to a steady-state dissociation constant in the nanomolar range, time for the separation procedure should not exceed 30–60 sec. Most investigators used a filtration assay, because of its unsurpassed speed, efficiency, and easy handling (Fleminger et al 1982; Maloteaux et al 1982; Bidart et al 1983; LeFur et al 1983; Shasken et al 1983; Itzchaky et al 1989). A pertinent example of the most dangerous pitfall of this technique is the stereospecific binding of radiolabeled opiates to glass-fiber filters reported by Pasternak and Snyder (1975). With radiolabeled spiroperidol only Fleminger et al (1982) mentioned a filter binding of 50% when using the most commonly employed Whatman GF/B filters. Other investigators seem not to have controlled for this phenomenon (Maloteaux et al 1982; Bidart et al 1983; LeFur et al 1983; Shaskan et al 1983; Itzchaky et al 1989). As demonstrated for various other receptor assays (Maayani and Weinstein 1980; Ito et al 1986) the apparent K_d and B_{max} values were not meaningful without coping with this phenomenon. In our experiments pretreatment of Whatman GF/C filters with 0.3% polyethyleneimine (Bruns et al 1983; Gundlach et al 1984) finally reduced unspecific filter binding to 5%–10% of total binding. Moreover, displaceable binding increased to 40%–60% of total binding and the experimental intraassay and interassay scattering decreased.

Conflicting results have been reported for the distribution of ^{3}H-spiroperidol binding sites on different subtypes of human peripheral blood mononuclear cells. Bidart et al (1983) reported that B lymphocytes had about double the capacity of T lymphocytes to bind this ligand, whereas Uzan et al (1981) found displaceable binding only on B cells. In agreement with Uzan et al (1981) we found displaceable binding only with unfractionated lymphocytes and in enriched B cell fractions. Because the amount of contaminating granulocytes contributes to a decrease in percentage of displaceable binding, especially in the low concentration range of radiolabeled spiroperidol, it is of critical importance to control for a constant composition of investigated cell suspensions. One possibility would be to investigate pure B cell suspensions, but this needs considerable additional methodological effort. If unfractionated cell suspensions are examined, it is essential to proceed immediately after blood drawing. Some groups did not refer to that (Bloxham et al 1981; Fleminger et al 1982; Bidart et al 1983; Rotstein et al 1983; Bondy and Ackenheil 1987; Feenstra et al 1989; Itzchaky et al 1989). For example, this might be one explanation for the failure of Feenstra et al (1989) to detect any displaceable binding in human lymphocytes, since this group proceeded 18 hr after venipuncture and did not control for granulocyte contamination.

The weak or missing stereoselectivity of (+)- and (−)-butaclamol in displacing ^{3}H-spiroperidol from its lymphocytic binding sites is a further topic of controversy. Bloxham et al (1981), Maloteaux et al (1982), Fleminger et al (1982), and Rotstein et al (1983) used ligand concentrations of 4 or 5 nmol/L in displacement experiments. Indeed, at these relatively high (>2 nmol/L) ligand concentrations, the stereoisomers of butaclamol exhibited no stereoselectivity. However, when low (< 1 nmol/L) concentrations of ^{3}H-spiroperidol were used in our experiments, (+)-butaclamol was about 8 times more potent than (−)-butaclamol (Figure 2b). This might be indicative of a specific binding site in the low concentration range of radiolabeled spiroperidol. But it is very unlikely that it resembles the central D_2 dopamine receptor, since (+)-butaclamol is about 1000 times

more potent than (−)-butaclaniol in displacing ^{3}H-spiroperidol from striatal D_2 dopamine receptors. Moreover, dopamine uptake inhibitors are ineffective at the neuronal D_2 receptor (Seeman 1981; Hitri et al 1991; our own data not shown).

Provided that these technical problems are considered, a final crucial step is the mode of data analysis. Rotstein et al (1983) and Itzchaky et al (1989) were not able to calculate any binding parameters and therefore used counts per minute (cpm) of displaceable binding for comparing schizophrenic with healthy subjects. Due to the atypically shaped saturation curve, various ways of calculation of B_{max} and K_d values have been used in the literature. It must be noted that none of the authors has statistically analyzed the model used. Our validation of these models by a reliable F test demonstrates that both the single-site model, which omits the nonsaturable part (ligand concentration range 0.05–3 nmol/L; Halbach and Henning 1989), and the complex model of one saturable and one nonsaturable site (ligand concentration range 0.03–3 nmol/L; Bondy and Ackenheil 1987) significantly improve the goodness of fit, and are preferable to the single-site model (ligand concentration range 0.1–5 nmol/L; LeFur et al 1983; Bidart et al 1983). The single-site model, which omits the nonsaturable part (ligand concentration range 0.05–3 nmol/L; Halbach and Henning 1989), is highly prone to bias, since the cutoff point is chosen arbitrarily. By using the model of two different binding sites, we were unable to achieve two K_d and B_{max} values in 10 of 12 individuals tested. Moreover, with concentrations of radioligand above 1 nmol/L only a linear nonsaturable increase in binding could be observed, in accordance with Feenstra et al (1989). As long as the molecular nature of the "spiroperidol binding site" is unknown, the complex model of one saturable and one nonsaturable site (ligand concentration range 0.03–3 nmol/L; Bondy and Ackenheil 1987) is the most adequate.

Based on these different calculation strategies, our B_{max} and K_d values in healthy subjects corresponded quite well to those of other research groups (Table 1). The remaining discrepancies might be explained by such pitfalls as variable composition of investigated cell suspensions (Bidart et al 1983; Bondy and Ackenheil 1987) and uncontrolled unspecific filter binding (LeFur et al 1983). Grodzicki et al (1990) analyzed the ligand concentration range 0.07–1.2 nmol/L only. This confirms the need to define exactly the details of experimental and mathematical procedures before comparing the results reported by different groups.

The molecular nature of the "^{3}H-spiroperidol binding site" is still unknown. Pharmacological characteristics are quite different from the central D_2 dopamine receptor with high (Bloxham et al 1981; Fleminger et al 1982; Maloteaux et al 1982) as well as low concentrations of ^{3}H-spiperone (see stereoselectivity of butaclamol-enantiomers). Moreover, cells have to be viable to demonstrate binding at all (Shaskan et al 1983; Feenstra et al 1989; Rao et al 1990; our own results), whereas neuronal D_2 sites can be detected in membrane preparations. Taken together with preliminary new results in our (Figure 2b) and other laboratories (Bondy et al 1990) with the specific dopamine uptake inhibitor GBR 12909, the "high-affinity site" resembles an active, energy-dependent transport site and not a true receptor. On the other hand, the possibility that this "binding site" might resemble one of the novel dopamine receptors ($D_{3,4,5}$; Sokoloff et al 1990; Van Tol et al 1991; Sunahara et al 1991) could not be definitely excluded. The "nonspecific, nonsaturable" part might represent an unspecific uptake into the cells (Fleminger et al 1982; Maloteaux et al 1983).

Apart from not exactly knowing the pharmacological and molecular nature of the "^{3}H-spiroperidol binding site" in peripheral lymphocytes, the findings in schizophrenia of

LeFur et al (1983), Rotstein et al (1983), Bondy and Ackenheil (1987), and Grodzicki et al (1990) have not been replicated (Bloxham et al 1981; Fleminger et al 1982; Maloteaux et al 1982; Shaskan et al 1983; Feenstra et al 1989; Rao et al 1990). The methodological issues discussed here might have contributed to this discrepancy.

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