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COMMENTARY

IN VIVO DOPAMINE RECEPTOR ASSESSMENT FOR CLINICAL STUDIES USING POSITRON EMISSION TOMOGRAPHY

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The aim of the present commentary is to discuss techniques to determine cerebral DA-receptor function *in vivo* and possible (future) applications of such techniques in clinical studies.

A brief summary will be given on existing approaches that are of particular clinical interest. The emphasis is laid on the *in vivo* receptor binding techniques, which have become of clinical interest with the advent of positron emission tomography. Both possibilities and limitations of the present methods to assess DA receptors and possible relationships to pathological or behavioral manifestations are described.

DA-receptor assessment methods

The possible involvement of dopamine (DA) receptors in pathology or in the clinical effects of psychotropic drugs has been recognized for more than 2 decades. Initially, the mechanisms of action of some psychotropic drugs were deduced from behavioral studies with rodents. For instance, drugs postulated to act as DA agonists enhance locomotor activity and induce certain non-functional types of behavior (stereotypy), when given intracerebrally or systemically [1–4]. DA-receptor antagonists, on the other hand, known in clinical practice as neuroleptics or antipsychotics, reduce locomotor activity and evoke an immobility (catalepsy) when given systemically [5–8]. When accurate biochemical assays for biogenic amines and their major metabolites became available, and turnover measurements of the amines were developed, it appeared that antagonists increase, whereas agonists decrease the metabolism of DA in most brain areas [9–13]. These drug effects are considered from the homeostatic point of view, as being able to overcome or reduce receptor blockade or stimulation, respectively, by concomitant changes in DA release, which may be directly or indirectly coupled to metabolism [14–17]. The *in vivo* actions of DA agonists and antagonists have also been recognized by, for example, a decrease or an increase in circulating prolactine, respectively, originating in the hypophysis [18–20].

A major breakthrough in receptor pharmacology includes measurements of direct receptor functions, such as cyclic AMP formation [21, 22], and of specific binding of DA agonists and antagonists, *in vitro* or *in vivo* [23–26]. With these techniques the heterogeneity of the DA receptors was established, the potency of drugs at the receptor could be quantified, and, finally, the localization and distribution of DA receptors in the brain were delineated [27–30]. Concerning the receptor heterogeneity, at least two subtypes of DA receptors have been described: D_1 receptors, which are coupled to adenylate cyclase activity and which have a low affinity for butyrophenones, and D_2 receptors, responsible for most behavior effects, inhibiting D_1-mediated cyclic AMP formation and having a high affinity for butyrophenones and benzamides [23, 31, 32].

Based on assays of metabolites in body fluids, circulating hormones, cyclic AMP formation or specific binding *in vitro*, several brain diseases have been associated with a dysfunction of DA neurotransmission.

Some clinical observations on DA receptors

Major dysfunctional neurotransmission of the DA neurons of the basal ganglia has been detected in Parkinson's disease, as was first reported by Hornykiewicz and coworkers [33–35], and was elaborated by several other groups by measurements of enzymes and metabolites in post-mortem brain tissue or body fluids [36–38]. Whether DA receptors in the basal ganglia are modified in Parkinson's disease, either before or during pharmacotherapy with L-DOPA or DA agonists, is a matter of controversy [39–41].

Low density of DA receptors (of the D_2 type) has been found in post-mortally obtained brain tissue of patients with supranuclear palsy [42, 43] and Huntington's chorea [44]. The latter could be expected from the apparent degeneration of medium size spiny neurons in the basal ganglia [45, 46], to which DA receptors are confined.

In vivo blockade of DA receptors during neuroleptic therapy is possibly manifested by the increase in cerebrospinal fluid and plasma levels of homovanillic acid and other DA metabolites [47–52] and prolactine [19, 20, 53–55].

Treatment with DA agonists (e.g. bromocryptine) evokes decreased circulating prolactine levels [19, 20, 56] and homovanillic acid [57].

All such *in vivo* approaches give semiquantitative data on the extent of DA receptor blockade or activation. For instance, it appears that only a few percent of the cerebrally formed DA metabolites enters the cerebrospinal fluid compartment, and that the brain area(s) that contributes predominantly is not known [56, 58].

During neuroleptic treatment, the density of D_2 receptors, as assessed by *in vitro* techniques,

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increases in rodents, but whether this occurs in humans is as yet uncertain [23]. Increased B_{max} values of D_2 receptors have been found in neuroleptic-treated patients [23, 59], but it has also been suggested that the B_{max} values were already high in the (schizophrenic) patients before drug therapy [60–62]. No change in post-mortem *in vitro* binding was seen with SCH 23390, a selective D_1 antagonist, in Parkinson's patients and neuroleptic-treated schizophrenics.

Increased B_{max} values may be present when these are extrapyramidal side-effects or tolerance towards side-effects. Biochemical evidence for the development of tolerance includes a diminished response to the neuroleptics of the DA system(s) as reflected by the metabolites in tissue or cerebrospinal fluid [50]. Attempts to relate pharmacokinetic aspects of antipsychotic drugs with clinical efficacy have revealed no clear dependency (e.g. Refs. 63–66). Such clinical correlative studies should be improved when the *in vivo* kinetics per individual are better understood, so that the relation to receptor occupancy can be deduced.

In vivo *receptor imaging*

Recently, techniques have become available to visualize specific binding sites of DA antagonists and other ligands (e.g. opiates, benzodiazepines and enzyme inhibitors) *in vivo* directly. In this commentary, the emphasis is on the visualization of cerebral DA receptors with antagonists, containing a positron emitting radionuclide. The distribution of such a radiopharmaceutical in the body and the brain can be determined by a reconstruction technique, positron emission tomography (PET). The principle and the possibilities of this technique for clinical studies have been reviewed recently in depth by several authors [67–71].

PET gives a quantitative distribution of radioactivity at a resolution of about 4 mm. Higher resolutions are not possible, because of the distance a positron moves through brain tissue, before annihilation. Two γ-quanta with opposing velocity vectors emerge, and these photons reach opposing detectors of a special PET-camera, enabling reconstruction of the line along which the annihilation had occurred, or, by taking into account the differences in travel time of the two γ-quanta, the site of annihilation. Often used radionuclides in DA receptor studies include ^{11}C or ^{18}F, having half-lives of 20.4 and 110 min respectively.

In vivo D_2 receptors have been visualized with several DA antagonists, including the butyrophenones 3-*N*-[^{11}C]methylspiperone [71–74], [^{18}F]spiperone [75, 76], *p*-[^{76}Br]- or [^{77}Br]bromospiperone (for positron and single photon emission tomography respectively [77–81]), [^{11}C]spiperone [82, 83], [^{11}C]pimozide [84, 85] and [^{18}F]haloperidol [86] and with the benzamide [^{11}C]raclopride [87].

Because of its quantitative nature, a major aim of present PET studies on receptors is to interpret PET images in terms of receptor characteristics, such as B_{max} and K_d (density and affinity constants of receptors respectively). Such a goal can be achieved only when the kinetic behavior of a radioactive ligand (often tracer doses) is precisely known, *in vivo*. Therefore, mathematical models to describe the pharmacokinetics of DA antagonists *in vivo* and to explain its specific binding in such terms have been developed. The underlying assumptions of any of these models (three have been described in a more definitive form, one in a preliminary form) will be discussed in detail below.

It should be realized that mathematical models are also required to understand *in vitro* binding data, and the assumptions are partly similar for *in vitro* and *in vivo* receptor binding models. In addition, with *in vivo* models, compartments such as blood and a relatively high and not purposely influenced non-specific binding compartment of the ligand have to be included. With PET only the total content of the ligand in a particular brain area can be measured and—in the case of clinical studies—it is often not possible to apply experimental designs to reduce or quantify non-specific binding. Moreover, in clinical approaches only a single—and often tracer—dose of the ligand can be applied.

Description of in vivo *models*

Thus far, published models [88–92] for the *in vivo* binding to dopamine D_2 receptors are based on three or four compartments in the region of interest (usually the basal ganglia):
compartment 1: the intravascular space with free ligand;
compartment 2a: the extravascular space with free ligand;
compartment 2b: the extravascular space with non-specifically bound ligand;
compartment 3: the extravascular space with specifically bound ligand.

The concept of compartments supposes that the ligand is evenly distributed within the compartment at any time so that every molecule of a particular ligand has the same chance to move from one compartment to another. Free ligand is considered to be in such a biochemical state that it has direct access to the specific binding sites (e.g. because it is dissolved in the aqueous phase of the tissue). Non-specifically bound ligand is confined to unsaturable tissue constituents and has no direct access to the specific sites; it has to change its biochemical state before binding to specific sites occurs (e.g. to move from the non-aqueous phase to the aqueous phase). As long as it is assumed that there is an immediate equilibrium between the free and non-specific binding states of the ligand and that the flux can be described by simple first-order kinetics, it does not matter that non-specifically bound ligand may actually be confined to several types of constituents. The compartments are represented in Fig. 1.

The differences between compartments 2a, 2b and 3 concern the biochemical states of the ligand. Therefore, the flow between these compartments can be expressed as a chemical reaction, whereas the flow between compartments 1 and 2a is physical. As a consequence, the distribution volumes of compartments 2a, 2b and 3 are assumed to be the same. Compartments 2a and 2b are often combined into a single compartment (2), because a fast equilibrium between the free and non-specific state of the ligand is assumed to occur. The amount or concentration

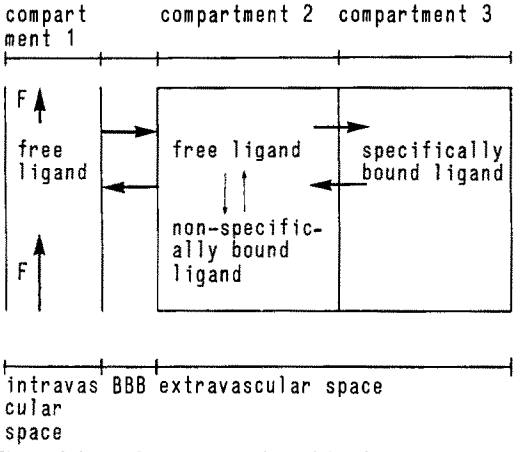

Fig. 1. Schematic representation of the three-compartment model, usually assumed in several published models. The big arrows denote the flow of the ligand; the little arrows express the equilibrium state between free and non-specifically bound ligand. Abbreviations: F, blood flow; BBB, blood–brain barrier.

of free ligand in compartment 2 can be expressed as a constant fraction of the total amount or concentration of the ligand in compartment 2. In most models, it is assumed that this fraction is the same in various brain regions.

In the thus far published models it is (tacitly) assumed that the ligand binds to non-interacting binding sites; thus, for example, positive or negative cooperativity is thought not to be involved. At a steady state the specific binding of a ligand can be expressed as:

$$[BL] = B_{max}/(1 + K_d/[L]) \quad (1)$$

where B_{max} = the maximum concentration of specific binding sites, $[BL]$ = the concentration of specifically bound ligand, $[L]$ = the concentration of free ligand, K_d = equilibrium dissociation constant = the ratio K_{off}/K_{on}, K_{off} = dissociation rate constant, and K_{on} = association rate constant.

Most of the models published thus far [88–91] use tracer doses of a ligand to avoid pharmacological side-effects. In these models the neuroleptic spiperone or analogues are used as a ligand because of their suitable properties [26, 75, 82, 88, 93, 94]. The dissociation rate constants (K_{off}) of spiperone and its analogues are very low; consequently, it takes a relatively long time before a steady state of specific binding has been reached, e.g. several hours for the rat brain [95]. Because of the relatively rapid radioactive decay of positron emitters, a steady-state analysis of the specific binding is not possible, so for PET only kinetic models with spiperone or its analogues have been developed [88–91, 96]. According to Perlmutter *et al.* [90], two types of models can be distinguished: the explicit model (or method) and the ratio model (or method).

The explicit model, first published by Mintun *et al.* [88], consists of a set of differential equations expressing the flow of ligand between the compartments in the striatum and the cerebellum. The cerebellum is assumed to lack specific binding sites for spiperone or its analogues. Solving these equations, analytically or numerically, leads to estimates of several model parameters. The set of differential equations for the cerebellum is simpler than the set of equations for the striatum because of the absence of specific binding sites in the cerebellum. The fraction constant, expressing the fraction of free ligand in compartment 2, is estimated in the cerebellum and used for the striatum. Some parameters, such as the regional cerebral blood flow (CBF) and the regional cerebral blood volume (CBV), are measured independently. Two important parameters, estimated by the explicit method, are K', which is approximately equal to $K_{on} \cdot B_{max}$, and K_{off}. Mintun *et al.* [88] define a new parameter, the "binding potential" (BP), so that $BP = B_{max}/K_d$. It can be derived from Equation 1 that, at a very low dose ($[L] \ll K_d$) and a steady-state situation for specific binding, BP approximates the ratio of specifically bound ligand over free ligand ($[BL]/[L]$) (Mintun *et al.* suggest erroneously that this is true at any dose). The model has been used for baboons. Computer simulations with the model demonstrate that errors in regional cerebral blood flow have little influence on the values of K' and K_{off}.

The ratio model of Wong *et al.* [89], although based on the same three compartments, has quite another structure. It is assumed that at a certain time after the administration of the ligand, but within the time of the PET measurements, steady state is reached for the flow of the ligand between the compartments except for the binding to the specific sites. Furthermore, it is assumed that K_{off} approximates zero. This last assumption means that the specific binding is considered to be irreversible and that a steady state is possible only if all the ligand is bound to the specific binding sites. Two other essential assumptions are that K_3 (the flow constant of compartment 2 to compartment 3) is much smaller than K_2 (the flow constant of compartment 2 to compartment 1) and that regional differences in cerebral blood flow and volume are negligible and, therefore, do not have to be incorporated in the model. An equation is derived in which the measurable ratio of radioactivity in the striatum over the cerebellum, minus unity, is equal to the product of the constant K_3 and the so-called "normalized integral" (NI). The normalized integral is defined as $F(t)/f(t)$, where $f(t)$ is the concentration of ligand in compartment 1 as a function of time and $F(t)$ is the integral of $f(t)$ for $t = 0$ to $t = t$. K_3 is exactly the same parameter as K' in the study of Mintun *et al.* [88]. Wong *et al.* [89] argue that the NI becomes linearly proportional with time and that, therefore, the ratio becomes linearly proportional to the time as well, because K_3 is a constant. This last relationship is indeed confirmed by PET studies [89, 90]. Thus, K_3 is proportional to the slope of the curve of the ratio of the striatal activity over the cerebellar activity against time. Hence, there is no direct estimate of K_3 but a value can be measured that is linearly proportional to it. When it is assumed that the proportionality factor is the same between individuals, then the different ratios at a certain time are indicative of differences of K_3 between the individuals.

A number of the underlying assumptions of these two models are summarized in Table 1. Important

Table 1. Comparison of several models with respect to their underlying assumptions and the estimated parameters of specific binding

	Assumptions (1–16) Estimated parameters (17, 18)	Mintun *et al.* [88]	Wong *et al.* [89]	Perlmutter *et al.* [90]	Wong *et al.* [91]	Farde *et al.* [92]	Swart *et al.**
1	Steady state for specific binding	n†	n	n	n	y	y
2	No specific binding sites in the cerebellum	y	y	y	y	y	y
3	Association of the ligand to a specific site is unimolecular‡	y	y	y	y	n	n
4	No interaction between specific sites	y	y	y	y	y	n
5	Dissociation rate constant (K_{off}) is zero	n	y	n	y	n	n
6	$K_2 \gg K_3$	n	y	n	n	n	n
7	$B_{max} \cong B_{max} - [BL]$	y	y	y	y	n	n
8	Equilibrium kinetics of non-specific binding	y	y	y	y	y	y
9	Fraction constant (f) is equal for various brain regions	y	—	y	n¶	—	n
10	No regional differences of permeability of the ligand through BBB	n	y	n	y	y	n
11	Flow of the ligand through the BBB is unsaturable	y	y	y	y	y	y
12	No metabolism of the ligand	y	y	n§	n§	y	n§
13	Metabolites do not pass the BBB	—	—	y	n	—	y
14	Metabolites do not bind to specific sites	—	—	—	y	—	—
15	No regional differences of blood flow	n	y	n	y	—	—
16	Intravascular amount of the ligand is negligible to the total tissue amount of the ligand per unit volume	n	y	n	y	—	y
17	Estimated parameters of specific binding with tracer doses	BP, K_{off}	K_3	BP, K_{off}	$\sim K_3$	BP	~BP
18	Estimated parameters of specific binding with pharmacological doses	—	—	—	K_3, B_{max},	K_d, B_{max}	$\sim K_d$, B_{max}

* Manuscript in preparation.

† Definitions: n, the assumption stated in the left column has not been made; y, the assumption stated in the left column has been made; (—), the assumption stated in the left column is irrelevant because of (an)other assumption(s); (~), proportional to; (≅), approximately equal to; K_{on}, constant of association to specific binding sites; K_{off}, rate constant of dissociation to specific binding sites; B_{max}, maximal concentration of specific binding sites; $[BL]$, concentration of specifically bound ligand; BP, B_{max}/k_d; K_3, $B_{max} \cdot K_{on}$; K_2, rate constant of flow of the ligand from compartment 2 to compartment 1; and BBB, blood–brain barrier; f, fraction of free ligand in compartment 2.

‡ This assumption is based on assumption 7.

§ Metabolism is assumed to occur only in compartment 1.

‖ A number of additional assumptions are made; see text.

¶ No fraction constant is proposed for the cerebellum; see text.

differences concern the following items: incorporation of the regional blood flow, the fraction of free ligand (f) in compartment 2, the inclusion of the intravascular concentration of the ligand in the total regional concentration, and the value of K_{off}. Neither model takes into account the influence of metabolites in the tissue, probably because it is known that spiperone and its analogues are poorly metabolized in the extravascular brain space [97, 98]. Recently, Arnett *et al.* [75, 94] demonstrated that metabolites of radiolabeled *N*-methylspiperone and spiperone appear rapidly in the circulation, that these metabolites hardly cross the BBB, and that there is, indeed, only minimal metabolism in the extravascular brain. In a recently published study, Perlmutter *et al.* [90] extend the models of Wong *et al.* and Mintun *et al.* for these metabolic features and compare them experimentally with PET studies of baboons, in considerable detail. It turns out that the incorporation of the metabolism in the circulation (but no passage

of the metabolites through the BBB) leads to other estimates of the parameters in both models. It appears that neglecting the fraction constant in compartment 2 in both brain areas influences the value of K' or K_3 rather strongly. Furthermore, it has been shown that the ratio of striatal activity over cerebellar tissue activity minus unity, is linear with time, but that the NI is not linear with time. As a consequence, the calculated values of K_3 vary with time, which is contradictory to its character as a constant. Perlmutter *et al.* state that this contrasting result may be caused by the erroneous assumption of $K_{off} = 0$ in the model of Wong *et al.* [89]. Other assumptions of Wong *et al.* [89] are confirmed by the study of Perlmutter *et al.* [90]: the small influence of regional differences in cerebral blood flow, the negligible amount of activity in compartment 1 as compared to the total amount in a brain region, and the much smaller value of K_3 as compared to the flow from compartment 2 to compartment 1.

Simultaneously with the study of Perlmutter *et al.* [90], Wong and coworkers published a modified version of the ratio model [91]. It has been adapted for metabolism in the circulation and for possible transport of metabolites through the BBB. Furthermore, it is assumed that these metabolites do not bind to the specific spiperone sites. The model has also been modified for binding to "secondary and non-D_2 dopamine receptors" in the striatum. The binding to these secondary sites is unsaturable and in equilibrium with the free ligand and, therefore, can be considered as a non-specific binding state of the ligand. No such non-specific binding exists for the cerebellum. The model is explicitly meant for irreversible binding because of the assumption of $K_{off} \simeq 0$. The authors applied the modified model to PET studies in human volunteers and showed that the NI becomes, at a certain time, proportional with time. Because the ratio is also linear with time, the parameter K_3 appears as a constant. This is in contrast with the results of Perlmutter *et al.* An important difference between the studies of Perlmutter *et al.* [90] and Wong *et al.* [91] is that the first authors used [^{18}F]spiperone, whereas the latter used [^{11}C]-*N*-methylspiperone. Another difference is that Perlmutter *et al.* [90] used baboons to compare the models, whereas Wong *et al.* [91] performed their studies in humans. It may be true that K_{off} is erroneously taken as equal to zero for spiperone in baboons, but that this assumption can be justified for *N*-methylspiperone in humans. In a further study, Wong *et al.* [96] tried to estimate B_{max} and K_{on} separately in the human brain with a modified model. The method has been based on preventing specific binding of [^{11}C]-*N*-methylspiperone ([^{11}C]NMSP) by pharmacological doses of the neuroleptic haloperidol. A number of additional assumptions have been made: e.g. the brain–blood partition coefficient and K_{on} are identical for [^{11}C]NMSP and haloperidol. In addition, the value of a parameter, resembling K_{off} for the human brain has been estimated with animal values.

The models described thus far are kinetic models, although some components are assumed to be in a steady state. Farde and coworkers [92] published a PET study with ^{11}C-labeled raclopride. From rat studies it is known that this ligand reaches a steady state for specific binding within an hour [99]. The measurements are analyzed with Equation 1, assuming that the kinetics of the ligand in the cerebellum are similar to striatal kinetics and that no non-specific binding occurs. By performing several PET scans with increasing doses on the same individual, they are able to construct a saturation curve, leading to separate estimates of B_{max} and K_d.

Evaluation of the in vivo *models*

Several criteria can be distinguished for evaluating these published models:

Accuracy of the models. The accuracy depends heavily on the underlying assumptions, which are based on conceptual grounds or are made to avoid too much complexity. The justification of the assumptions should be ideally based on experimental data but this is not always possible. Other ways of testing the assumptions are by comparing different models or versions of models with the same set of data (as Perlmutter *et al.* [90] did with modified versions of the models of Mintun *et al.* [88] and Wong *et al.* [89]) and by simulations of a model testing the sensitivity of model parameters by changing one of the parameters (e.g. Mintun *et al.* [88]).

A number of assumptions of the models thus far published are not considered critical. Without clear arguments it is assumed that specific binding sites of spiperone or its analogues do not have interacting features *in vivo*. Experiments with different species [100, 101] demonstrate that spiperone sites are independent *in vitro*. But this may not be true for the *in vivo* situation because the micro-environment of the specific sites is quite different and may lead to other results [102, 103]. Except for the study of Farde *et al.* [92] no tests have been performed to check this assumption. Another assumption which may be wrong is the similarity of the fraction constant, expressing the fraction free ligand in compartment 2, in different brain regions [88, 90]. Some authors do not distinguish such a fraction constant at all [89, 92], tacitly assuming that all the extravascular ligand has direct access to the specific sites. It can be derived that this assumption does not influence the estimation of B_{max} but leads to another value of K_{on}. The modified ratio model of Wong *et al.* [91] contains such a fraction constant for the striatum. It is defined, however, as binding to non-dopaminergic receptor sites. Consequently, the ligand is (tacitly) assumed to have access to the receptor from the aqueous phase as well as from the non-aqueous phase, and no fraction constant is assumed to exist in the cerebellum (because of the absence of specific spiperone binding sites).

Recently, we constructed steady-state models for the *in vivo* binding of spiperone in rat brain to test some of the underlying assumptions made in already published models*. Our models were fitted with experimental data from rat striatum, frontal cortex and cerebellum. It appears that the kinetics of spiperone in the frontal cortex are similar to those in

* J. A. A. Swart *et al.*, manuscript in preparation.

the cerebellum, whereas the striatal kinetics are not similar to it, due to differences in the passage of the ligand through the BBB or to different fraction constants in compartment 2. The existence of regional differences in the rat brain of the pool of non-specifically bound spiperone has also been emphasized by Laduron *et al.* [26] and Barone *et al.* [104]. Another result is that spiperone binding in the frontal cortex is indeed non-cooperative, whereas the striatal specific binding shows interaction features, an indication of more interacting sites on one receptor molecule. This challenges one of the most basic assumptions of most of the models published thus far. If this is true, then it is possible that ligands may differ in respect to their binding to different sites resulting in, for example, different B_{max} values. This idea is supported by the finding that *in vivo* spiperone binding has a B_{max} value twice that of the dopamine agonist *N*-propylnorapomorphine in some brain areas of the rat with a high density of D_2 receptors [105–107].

Estimated parameters of the model. As shown in Table 1, none of the models generates a value of B_{max} and K_{on} or K_d separately, when tracer doses are used. Separate estimation is theoretically possible when pharmacological doses are used [92, 96]. Mintun *et al.* [88] stated that "with the administration of radioligands in tracer amounts, no *in vivo* model can separate the receptor's forward rate constant from B_{max}". This statement is not exactly true because Wong *et al.* [96] measured B_{max} separately, using a tracer amount of radiolabeled *N*-methylspiperone and pharmacological amounts of non-labeled haloperidol. Separate estimates, however, will probably be very difficult without the use of non-tracer amounts of a ligand. Mintun *et al.* [88] proposed using the binding potential (B_{max}/K_d) as the parameter for *in vivo* binding features, because it measures the potential for specific binding: when the B_{max} is higher and/or the K_d is lower for a particular ligand as compared to another one, more of the ligand will be specifically bound. We could derive that the ratio of striatal over cerebellar activity at a tracer amount of the ligand is linearly proportional to the BP plus a constant when a steady state has been reached*. It depends on the aims of clinical practice whether such combined parameters are useful. The same is true for estimated values which are proportional only to a wanted parameter.

Practical usefulness. Some models require much more effort to be applied than others. The models of Wong *et al.* [89, 91] require one or a few PET scans, with a single dose, whereas the models of Mintun *et al.* [88] and Perlmutter *et al.* [90] require, besides the PET scans with the neuroleptic, PET scans for measurement of local cerebral blood flows and blood volumes. Measurements of B_{max} separately from other parameters require pharmacological amounts of neuroleptics and/or multiple PET scans of the same person. Medical ethical problems may arise because of the radioactive dose when more studies are performed or because of side-effects of pharmacological doses.

None of the models fulfills all the criteria satisfactorily. Both models of Wong *et al.* [89, 91] have more rigid assumptions than the models of Mintun *et al.* [88] and Perlmutter *et al.* [90]. The latter, however, are more complex and less practical, and are only experimentally used for baboons. Most of the models require special features of the ligand (e.g. $K_{off} = 0$ in the models of Wong *et al.*), which limit the application of the models.

At present, no model for general clinical practice is available. Modeling the *in vivo* assessment of properties of the neuro-receptor, however, is a new branch, and new models and approaches may be expected. Probably, not the use of a single model but rather a combination of different types of models for various types of ligands (agonists and antagonists) will be a better strategy for assessing the behavior of neuro-receptors.

Requirements for specificity

The specificity of *in vivo* binding can be established, in essence, similarly to that of *in vitro* binding. Saturation, prevention, or displacement of specific binding can be determined by coadministration of the labeled ligand with carrier doses and by the pretreatment or treatment with drugs of various specificities respectively. As a rule, there should be a close relationship between the distribution in the brain of specific *in vivo* binding and the density of binding sites, as determined with *in vitro* methods, although exceptions may occur [108].

The *in vivo* binding should be confined to particular neuronal (or glial) elements, which can be eliminated by lesions. For example, after lesioning of the striatum with kainate, the *in vivo* binding (as has been shown earlier for *in vitro* binding) of spiperone and *N-n*-propylnorapomorphine (NPA) had diminished by more than 60% [105, 106]. No change in the *in vivo* binding of these ligands was seen after destruction of the ascending DA pathways, indicating a lack of involvement of DA autoreceptors [103, 105, 106]. After long-term administration of the neuroleptic haloperidol, higher DA-receptor density was found in the rat striatum with *in vivo* binding, as has been shown several times previously with *in vitro* techniques [109]. However, in the latter study specific tracer binding was not increased. Moreover, there was no clear-cut linear relationship between *in vivo* determined B_{max} values and specific tracer binding in various brain regions [107]. Specific binding of the ligand depends on the state of the ligand in the extravascular compartments (compartment 2, Fig. 1). We observed that specific binding of tracer amounts of spiperone was increased in kainate-intoxicated striatum, despite a reduced B_{max} [106], which could be attributed to an increase in the concentration of the free ligand. All these studies imply that with tracers, as normally applied in PET studies, receptor density cannot be deduced easily from radioactivity distributions (see also previous sections).

An additional requirement for *in vivo* binding studies, which almost never can be met using *in vitro* techniques, is that of a close relationship between receptor occupation and pharmacological response. In the case of D_2 receptors, such a relationship was

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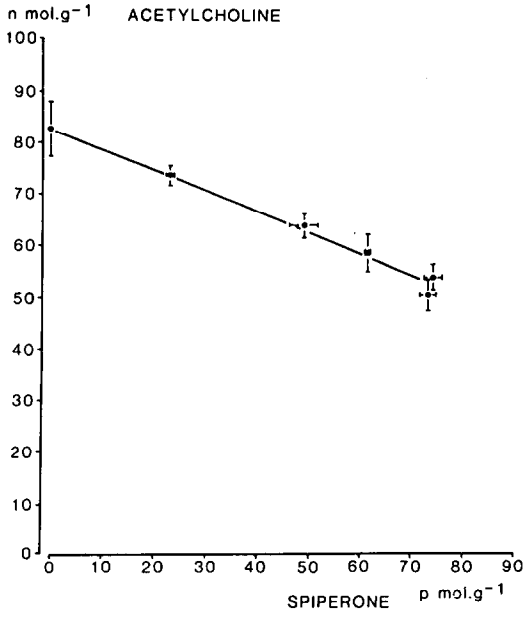

Fig. 2. Relationship between DA-receptor (D_2 type) occupancy by spiperone and acetylcholine levels in the rat striatum. A simple linear relationship was obtained, indicating that all binding sites have the characteristics of the functional receptors. Data are derived from Ref. 110. Bars indicate SEM of three to nine observations.

determined between striatal spiperone binding and the decrease in acetylcholine levels [110] (Fig. 2). Thus, at maximal occupation of the binding sites the striatal levels of acetylcholine were maximally decreased, indicating that spiperone did not occupy receptors with a lower affinity, without a direct relationship to cholinergic processes [110]. After denervation of the striatal or dopaminergic input, such relationship was lost, showing that a receptor occupied by the antagonist was only effective on the cholinergic neurons in the presence of DA. This indicates that under normal function of the DA system most of the DA receptors are occupied by the transmitter [109].

During long-term neuroleptic treatment, the relationships between D_2-receptor occupation by spiperone and acetylcholine levels were maintained, although tolerance was evident. Again, in the absence of DA input, no relationship between D_2-receptor blockade and cholinergic processes was evident [109].

With the agonist NPA no direct relationships between receptor occupation *in vivo* and acetylcholine level or DA metabolism in the rat striatum were found. Either a subfraction of the DA receptors (e.g. autoreceptors) was involved in some of the biochemical effects, or the drug does not behave as a full agonist *in vivo* (see discussion in Refs. 105 and 106).

Attempts have also been made to relate behavioral effects directly to receptor occupation. For instance, a degree of catalepsy in rodents is produced by doses of neuroleptics (e.g. spiperone) that also activate tyrosine hydroxylase in the striatum [111].

The dose dependency of some neuroleptics in preventing specific tracer [^{3}H]spiperone binding and increasing DA metabolism or apomorphine-induced hyperactivity and stereotypy was determined in rats [112]. This study emphasizes that *in vivo* [^{3}H]spiperone displacement does not correlate in a simple way to any of the responses.

However, behavior is a reflection of a more integrated function of an organism and often cannot be directly deduced by the activity of a class of receptors. So, catalepsy in rodents is determined not only by the striatum (e.g. DA receptors) but by other brain areas as well [112–114]. In addition, peripheral mechanisms, such as those of the adrenal medulla, may contribute [115]. Behavioral tolerance to neuroleptics, e.g. diminished cataleptic response to a challenge dose of a neuroleptic, is seen after long-term neuroleptic pretreatment. Biochemical tolerance is manifested by an increase in receptor number and by a decrease in the effects of a challenge dose of a neuroleptic on DA metabolism and cholinergic processes. It has often been suggested that the biochemical and behavioral responses are related. This, however, does not appear to be the case. Thus, lack of behavioral tolerance could be induced, whereas from a biochemical point of view rats were tolerant [116, 117]. Development of behavioral tolerance is dependent not only on the biochemical state of the brain, as induced by the drugs, but also on environmental influences. In any case, a direct correlation between the state of cerebral receptors and behavior cannot be expected when environmental factors contribute or predominate. This is particularly relevant for clinical studies because of great environmental influences on human behavior.

Concluding remarks and summary

Several clinical studies emphasized the importance of cerebral DA receptors for the understanding of either pathologic or pharmacologic effects or both. Relevant hypotheses are based on the chemical examination of body fluids or post-mortem brain tissue, either of which gives rather indirect indices for receptor function, at best. A definitive way to improve our understanding of DA receptor function is to assess specific binding parameters *in vivo*.

Application of positron emission tomography allows us to study cerebral DA receptors *in vivo* and thus to relate receptor state and behavior. In addition to adequate instrumental facilities (which are not the subject of this commentary), useful radiopharmaceuticals and validated mathematical models to describe *in vivo* specific and non-specific binding are required. These models should enable us to derive meaningful physiological parameters from a limited number of PET measurements, preferentially obtained with tracer doses. As yet such requirements have not been met.

A further possibility of *in vivo* binding approaches is to relate directly receptor occupancy and effects, defined in terms of either physiological changes or behavior. Apart from a few exceptions, such studies concerning DA receptors have not yet appeared.

The nature of the presently used ligand is still limited to antagonists. No PET studies with DA agonists have appeared. Furthermore, little is known

about the pharmacokinetics and the state of the ligands in various brain compartments, in normal and pathologically disturbed brain tissue. In particular, the consequences for receptor binding in such conditions should be studied further.

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Note added in proof: Recently a kinetic model for [^{18}F]-spiperone has appeared which emphazises (a) peripheral metabolism, (b) differences in kinetics between the striatum and the cerebellum, and (c) the occurrence of a few specific binding sites in the cerebellum [118].

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