Abstract

Recently, two research groups published numbers for D₂ receptor sites in the neostriatum of drug-naive schizophrenic patients, obtained in vivo by positron emission tomography (PET). One study appeared to confirm the increase of D₂ receptor numbers, while the other study did not. A workshop was convened in Montreal to examine the reasons for the discrepancy between the results obtained by the two groups. The workshop considered patient populations, PET instrumentation and scanning methods, pharmacology, and modeling. The workshop identified differences between the approaches of the two groups that could contribute to the divergent results, including age and chronicity of the patient samples, brain region selected for study, metabolism of the different radioligands in blood and brain, reversibility of binding, PET instrumentation, and complexity of data analysis. The workshop concluded that these initial efforts had made considerable progress in establishing the role of PET in the understanding of the biochemical processes underlying mental illness. In particular, the unique ability to quantify regional neuroreceptor density at different stages in the evolution of the disease has been implemented. At the same time, the work so far and this conference served to identify the main sources contributing to the different findings from the two centers. This information will be important in designing the next phase of the research which will build upon and reconcile these apparent discrepancies.

The dopamine hypothesis is at present the most widely accepted theory concerning the neurochemical abnormalities that may occur in schizophrenia. It postulates that patients suffering from schizophrenia have an apparent overactivity of dopaminergic mechanisms in crucial brain regions. The dopamine hypothesis is supported by several lines of evidence, including the mechanisms of action of neuroleptic drugs and the fact that dopamine agonists tend to exacerbate the symptoms of schizophrenia (Andén et al. 1964; Van Rossum 1967; Seeman et al. 1976; Creese et al. 1978).

If a characteristic abnormality in the dopamine system exists in schizophrenia, it could be at any of a series of points in a cascade of events involved in dopamine transmission: transmitter synthesis, neuronal activation and release, metabolic breakdown, reuptake, or sensitivity of postsynaptic dopamine receptors. These various steps have been analyzed in a variety of studies, and the bulk of the evidence at present appears to suggest (although far from conclusively) that the abnormality involved could be an excessive number of dopamine type-2 (D₂) receptors (Lee and Seeman 1977;
Kleinman et al. 1982; Seeman et al. 1984). The evidence is based on the study of post-mortem brains, in which increased numbers of D₂ receptors have been observed. Since the principal mechanism of action of neuroleptic drugs is to block D₂ receptors, a major concern in such post-mortem research has been that the increased numbers of D₂ receptors could be a result of proliferation induced by chronic blockade. In post-mortem samples, never-treated patients are necessarily quite rare, and sample size in these post-mortem studies has not been sufficient to resolve the issue.

As techniques for in vivo imaging of neuroreceptors have emerged during the past several years, it has become clear that this imaging modality offers a special opportunity to determine whether a D₂ receptor increase in schizophrenia could explain at least a portion of its pathogenesis. The logical strategy in such work is to recruit a sample of young schizophrenic patients who have never been treated with neuroleptic medication and to measure D₂ receptor density in their brains.

During the past few years, two major research groups have been working toward this goal: a team at the Karolinska Institute in Stockholm and a team at the Johns Hopkins Medical Institutions in Baltimore. The scientific community has eagerly awaited the results of these studies, since the studies have the potential to yield important evidence concerning the neural mechanisms of schizophrenia.

In late 1986 and mid-1987, these two research groups published data concerning $B_{\text{max}}$ (a measure of receptor density) for D₂ receptor sites in the neostriatum of drug-naive schizophrenic patients (Wong et al. 1986c; Farde et al. 1987b). The results from the Hopkins team seemed to confirm the existence of increased $B_{\text{max}}$ in schizophrenia, while the results from the Karolinska team did not. The data published by the two groups are summarized in table 1. Both groups had also previously published normal values (Farde et al. 1986; Wong et al. 1986b), as shown in table 1, which includes all the values published by the two groups. The Hopkins results indicate a twofold to threefold increase in D₂ receptor density for schizophrenic patients (drug treated and drug-naive) as compared to the control group, while the Karolinska team finds no significant difference between controls and drug-naive patients. The Karolinska team has not yet reported values for drug-treated patients.

Because of the enormous importance of understanding the neurochemical mechanisms involved in schizophrenia, as well as the capacity of position emission tomography (PET) scanning to study human brain chemistry and its abnormalities in vivo, it became clear that it was important to attempt to determine the reasons for the differences as quickly as possible. Consequently, a workshop was convened in Montreal to discuss the possible reasons for, and implications of, the discrepancy and, if possible, to recommend steps that may be taken to resolve the discrepancy. It was not the intention of the participants to address the merits or demerits of the dopamine hypothesis or the power of PET scanning to explore brain chemistry. The value of both is well recognized. The major purpose of the workshop was to examine the reasons for the discrepancy between the results of the two groups and to build on their current methodological achievements to recommend ways that PET scanning can continue to be applied to advance our knowledge about the neural mechanisms of schizophrenia.

The workshop considered the differences between the studies under four headings: Patient Populations, PET Instrumentation and Scanning Methods, Pharmacology, and Modeling. For each of these subdivisions, the representatives of the two teams presented their methods and viewpoints briefly, and the presented material was then submitted to general examination.

### Table 1. $B_{\text{max}}$ and $K_d$ for dopamine D₂ in normal volunteers and in drug-naive and drug-treated schizophrenic patients

<table>
<thead>
<tr>
<th>Authors</th>
<th>Subjects</th>
<th>$B_{\text{max}}$ (pmol $g^{-1}$)</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farde et al. (1986)</td>
<td>Normals</td>
<td>14 ± 2</td>
<td>4</td>
</tr>
<tr>
<td>Wong et al. (1986b)</td>
<td>Normals</td>
<td>9 ± 1</td>
<td>4</td>
</tr>
<tr>
<td>Wong et al. (1986c)</td>
<td>Controls</td>
<td>17 ± 3</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Drug-naive patients</td>
<td>42 ± 5</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Drug-treated patients</td>
<td>43 ± 5</td>
<td>5</td>
</tr>
<tr>
<td>Farde et al. (1987b)</td>
<td>Controls</td>
<td>25 ± 6</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Drug-naive patients</td>
<td>25 ± 7</td>
<td>15</td>
</tr>
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</table>
and discussion by the group as a whole. At the end of the day, a further general discussion of conclusions and recommendations occurred. This report summarizes the results of the workshop.

The basic facts of the studies are as follows. The team from the Karolinska Institute used a new highly selective D₂ antagonist, raclopride, labeled with carbon-11 (¹¹C). Although a complete kinetic analysis has not yet been published, this compound appears to equilibrate rapidly, and therefore an equilibrium distribution model based on the familiar Michaelis-Menten kinetics was applied to measure \( B_{\text{max}} \) and \( K_d \) in striatal regions. To establish a Scatchard line, two PET studies at different dose levels were required. The maximal number of occupied sites (\( B_{\text{max}} \)) and the equilibrium dissociation constant (\( K_d \)) could thereafter be calculated from the known specific activity of the tracer. In each study, the time-activity curve (TAC) of the radioactivity accumulated in the cerebellum was assumed to represent the unbound tracer in brain.

The team from Johns Hopkins determined the number of D₂ sites using a dynamic, nonequilibrium application of the Woolf receptor analysis method (Haldane 1957). This method derives the maximum number of sites from the slope of the linear relationship between free/bound ratios plotted against the concentration of free ligand, using haloperidol as a saturating agent and \( ^{11} \text{C} \)-labeled methylspiperone as the tracer of receptor saturation. Unlike raclopride, the binding of methylspiperone is irreversible in the short period of time available for scanning of \( ^{11} \text{C} \)-labeled compounds (the half-life of \( ^{11} \text{C} \) is 20 minutes). The unbound \( ^{11} \text{C} \)-methylspiperone was determined from the brain-blood partition coefficient, rather than from the cerebellar TAC alone. The method can only be used to calculate \( B_{\text{max}} \), when the rate of association of methylspiperone is known.

Both groups studied drug-naive patients to eliminate the effects of previous treatment on the dopamine system. Both chose to use the neostriatum, a site rich in D₂ receptors, because the large number of receptor sites in this region is well established and permits maximally precise quantification with current PET methods. While some forms of the dopamine hypothesis postulate D₂ abnormalities in the limbic system rather than the striatum, other evidence implicates the importance of the basal ganglia (Seeman et al. 1984; Early et al. 1987; Gur et al. 1987). It is well recognized that patients with basal ganglia disease, such as Huntington’s or Parkinson’s diseases, manifest some symptoms related to those observed in schizophrenia. Also, the neostriatum including the nucleus accumbens is the only major area of specific binding to D₂-dopamine receptors in brain.

**Patient Populations**

The subjects collected by the Karolinska and the Hopkins groups differ in several important ways.

The Karolinska group collected a relatively young sample. The mean age was 24 ± 4 years (\( n = 15 \)). Of the 15 patients, 13 met DSM-III (American Psychiatric Association 1980) criteria for schizophrenia or a schizophreniform disorder at the time that they were ascertained. In two patients, the disease was relatively acute at time of study. Of those patients who had not had a 6-month duration of illness at the time of entry into the study, all have been evaluated with subsequent interviews and now meet DSM-III criteria for schizophrenia (i.e., they have all been ill for at least 6 months). Diagnostic assessment instruments included the Brief Psychiatric Rating Scale (BPRS; Overall and Gorham 1962) and the Åsberg et al. Rating Scale (Åsberg et al. 1978). Diagnoses were confirmed by conversations with an available family member whenever possible, as was the absence of a past history of neuroleptic treatment. The Swedish group dated duration from the time that the first prodromal symptom was noted and estimated the mean duration of illness of their sample to be 1.9 years. At the time of scan, all patients either had delusions and/or hallucinations, or had positive formal thought disorder (\( n = 6 \)). Negative symptoms were not rated. Two had abnormal findings on computed tomography (CT) (one with ventricular enlargement, one with a calcification in the pallidum). Patients were kept in the hospital from a few days to 2 weeks before scanning. To obtain their sample of 15 drug-naive patients, the Swedish group evaluated a total of 17 patients, 2 of whom refused. Among the 15 who agreed to enter the study, all were able to complete the scanning protocol. Their controls were matched in sociodemographic status and were comparable in education. They were somewhat older than the patients, with a mean age of 29 ± 5 (\( n = 14 \)) years.

The Hopkins sample was somewhat older and more chronic. The
mean age of the patients was 31 ± 4 (n = 10). The Hopkins group estimated duration of illness from the time of onset of the first psychotic symptoms. In spite of this narrower definition, their average duration of illness was 4.7 years. All patients met DSM-III criteria and therefore were ill for at least 6 months. A number of these patients were drug-naive because they had refused treatment in the past. Most were ascertainment through the Johns Hopkins Hospital emergency room and scanned within 48 hours of ascertainment. The controls in the Hopkins study were younger than the patients. Their mean age was 24 ± 2 years (n = 11). Many of the controls were hospital personnel and therefore were not closely matched to the patients on education and socioeconomic status. Evaluation instruments included the BPRS (Overall and Gorham 1962), the Mini Mental Status Exam (Folstein et al. 1975), the Present State Examination (Wing et al. 1974), the Scale for the Assessment of Negative Symptoms (Andreasen 1983), and the Hamilton Rating Scale for Depression (Hamilton 1960). All patients had a previous CT scan, and all scans were normal. The Hopkins group also confirmed diagnosis, symptomatology, and drug-naive status by conversations with an available family member whenever possible. Among the patients ascertained, approximately 60 percent agreed to participate, and 60 percent of these were able to complete the protocol.

Thus, the two groups began initially with different sampling strategies. The Hopkins group elected to use a narrow definition and to identify a sample with considerable chronicity. They studied patients who have been ill a relatively long period of time. The Karolinska group, on the other hand, elected to study relatively acute cases.

The differences in phenomenology and age could conceivably account for some of the differences in the findings of D2 receptor density. This is possible for several reasons. First, many investigators who work with schizophrenia suspect that this illness is heterogeneous. Any sample of patients with schizophrenia may comprise several subpopulations that differ in clinical characteristics, pathophysiology, and etiology. For example, Seeman et al. (1984) identified a bimodal distribution of receptor densities in drug-free schizophrenic patients. Since the two groups did not differ in their clinical characteristics, it is interesting that their pathophysiology was different.

A second, and somewhat related, explanation is that these two groups of patients may represent different stages of the illness. The Karolinska sample consists of individuals who have been ill a relatively short period of time, while the Hopkins sample is both older and more chronic. If receptor density increases during the course of the illness, then the findings may be explained by differences in chronicity. Nevertheless, we recognize that we know very little about the actual relationship between functional dopamine activity in the brain and the clinical phenomenology and course of schizophrenia, and the postulated relationship between functional dopaminergic excess and positive symptoms requires further study.

A third possible explanation for the difference in findings is related to the difference in ages in the two samples. The Hopkins patients are considerably older than the Karolinska patients, while the Hopkins controls are relatively younger and the Karolinska controls are relatively older. Wong et al. (1984) have observed a decrease in \( B_{\text{max}} \) with increasing age in normal individuals. The average age of the Karolinska normal controls was 29 ± 5 years, whereas that of the Hopkins controls was 24 ± 2 years. This slight difference in age is probably not sufficient to explain the findings on the basis of a decreased number of receptors in the Karolinska normals as compared to the Hopkins controls. The difference in age between the Karolinska versus the Hopkins patients is more substantial (24 ± 4 vs. 31 ± 4). If the curve for decrease in receptor density is the same for schizophrenic patients as for normal subjects, then this difference in age would produce a result opposite to that actually obtained and therefore would not explain the discrepancy in findings between the two groups. If the number of receptor sites falls with age, the higher age of the Hopkins patients would tend to reduce the probability of a significant difference. The reverse is true of the Karolinska sample. An additional explanation concerned the nutritional status of the patients. The patients in the Swedish sample appear to have been living in the context of a relatively normal environment, while at least some of the Hopkins patients may have been more impoverished. However, a review of general medical status of the Hopkins sample revealed no significant plasma abnormalities. Furthermore, the Hopkins subjects with the highest \( B_{\text{max}} \) values were completely normal nutritionally.

Taken together, the differences in the patient and control samples
were substantial. Our current level of knowledge concerning the relationships between dopamine function, clinical phenomenology, course of illness, and possible heterogeneity is not sufficient to make a definitive interpretation of the effect of these differences in sampling, and these issues clearly require further study. Nevertheless, it seems possible that sampling differences could account for a portion of the discrepancy.

**PET Instrumentation and Scanning Methods**

This section addresses the differences in scanning procedures employed by the Karolinska and Johns Hopkins groups. Of particular concern is the extent to which those differences contribute to the contradictory findings of the two groups with regard to the density of dopamine D2 receptors in the striatum of normals and drug-naive schizophrenics. It is important to note that we are more concerned with the comparison between the two subject groups, normals and schizophrenics, within each center than in the absolute differences in receptor density measured at each center for nominally equivalent groups. The receptor ligands and experimental methods employed by the two groups are quite different, but they are internally consistent. If they do indeed measure the same receptor populations, they should not produce a twofold to threefold difference in the ratio of $B_{max}$ for schizophrenic versus normal subjects.

Studies at the Karolinska Institute were performed on a Scanditronix PC-384, a seven-slice machine with a transverse resolution of 7.6 mm and an axial resolution of 11.5 mm in direct slices (8.0–11.1 mm in cross slices). The software package contains an explicit deconvolution procedure to correct for scattered events, a projection thresholding procedure for attenuation correction, and a singles-based correction for randoms and dead-time.

A head-fixation system made it possible to transfer the subject's positioning from CT to PET. Regional identification was performed by outlining the putamen and head-of-caudate on CT. The regions of interest were drawn on the CT images and transferred to the PET images. Anatomical planes were parallel to the canthomeatal plane, and the PET plane containing the striatum was chosen to exhibit minimal partial volume errors. The $B_{max}$ values published by Farde et al. (1987b) were for the putamen, and they stated at the workshop that the caudate gave similar results.

Studies at the Johns Hopkins Medical Center were performed using a CTI NeuroECAT in high-resolution mode. Three direct slices are obtained, separated by 32 mm, with a transverse resolution of 8 mm and an axial resolution of 14 mm. Cross slices are not collected with the collimators in place. No scatter correction was performed, and attenuation correction was performed using the software "ellipse" approach. The attenuation coefficient was assumed to be 0.088 cm$^{-1}$, an empirical value which is less than the narrow beam attenuation coefficient of water (0.096 cm$^{-1}$) because of the presence of small-angle scattered events in the acceptance angle of the detector pairs. Random events were removed during acquisition using an "off-time" window. Regional identification was also performed with the aid of CT. However, the head-fixation system did not allow the transfer of positioning from CT to PET. A set of 8 mm thick slices, separated by 3 mm, was obtained and the slice which contained the maximum caudate area was chosen. The calcified pineal gland was always observed in this CT slice. Regional values were obtained by placing a 2 x 2 pixel square over the head of the caudate on the PET scan slice.

The different physics/imaging methods employed by the two centers leave ample room for systematic differences in absolute $B_{max}$ values caused by different estimates of striatum and cerebellum radioactivities. For example, the explicit removal of scatter in the Karolinska machine results in greater contrast between the striatum and surrounding low-activity regions. Also, the choice of anatomical plane is somewhat different and the axial resolution of the Hopkins machine is 15 mm, compared to 11.5 mm of the Karolinska system. This would result in lower striatal values in the Hopkins sample if identical regions of interest (ROIs) were used. However, the Hopkins group used a peak-picking strategy to obtain their TAC data, whereas the Karolinska team used larger anatomically defined ROIs for putamen and head-of-caudate. The Hopkins approach would tend to offset any partial volume differences because the "smearing" of radioactivities must decline when regions become larger if the radioactivity is uniformly distributed in a region. As stated initially, however, none of these observations explain the intra-center difference between schizophrenic and normal subjects observed in Baltimore but not in Stockholm. A
characteristic change in caudate size in schizophrenic patients might affect the results from the NeuroECAT more than from the PC-384. For a given axial extent of caudate the PC-384 is closer to full signal recovery, and any such size change will affect the apparent signal to a lesser degree. For the observed results to be attributable to this hypothetical phenomenon, the caudate would have to increase in size for schizophrenic patients. Wong et al. (1986c) found no evidence for systematic size differences in the caudate.

A potential source of discrepancy is the fact that the Karolinska results were obtained from the putamen, while the Hopkins results were obtained from the caudate. However, similar results have later been noted for the putamen by the Hopkins team (Wong et al., personal communication).

When considering only physics and imaging, it is difficult to identify any artifact that would give rise to the result apparent in figure 1 of Wong et al. (1986c) where the putative increase in $B_{\text{max}}$ for schizophrenic patients is illustrated by a large difference between a normal and a schizophrenic subject for haloperidol blockade of $D_2$ receptors. However, such images can be misleading if proper normalization is not carried out such that non-involved cortical areas are scaled to equivalent intensities. D.F. Wong added that the distinction is not typical of the two populations and that the measured differences are more apparent in the fitted parameters of the TAC data than in a comparison of the raw activity images. In this case we must consider the form of the TACs which generate the observed difference in the uptake rate constant $k_3$ (proportional to $B_{\text{max}}$; see Modeling section) between the subject groups and how subtle differences in scanning procedures may affect the $B_{\text{max}}$ measurements. With no blocking dose of haloperidol, both groups show a steep slope of the linear phase of the theta plot (normalized TAC data plotted against normalized plasma integral [Gjedde 1981, 1982]). After haloperidol administration, both groups show slopes much closer to zero. A small difference in the slope between the two subject groups could give rise to a larger ratio between the $k_3$ values obtained from kinetic analysis. Hence the Hopkins model is likely to be sensitive to small differences, both real and artificial, between the two subject groups. If the Hopkins results are to be judged as artificial, one must invoke a mechanism that would affect the schizophrenic patient differently from the normals, such as a different ligand distribution giving rise to a different, and improperly corrected, scatter distribution. While possible, such speculations seem tenuous.

### Pharmacology

A major difference between the methods of the two centers is the use of different ligands for the dopamine $D_2$ receptors. Aspects of these ligands and their receptors relevant to the studies under scrutiny were discussed (see table 2). The essential questions relate to whether the association rate of methylspiperone can safely be inferred from the $IC_{50}$ of haloperidol, and whether accurate haloperidol concentrations in brain can be calculated from the steady-state partition of tracer methylspiperone and serum haloperidol. The identity between blood-brain partition coefficients for tracer methylspiperone and tracer haloperidol in vitro was reported by D.F. Wong at the workshop. The Hopkins procedure, on the other hand, does not require accurate measurements of specific activity, as long as the actual injected dose of

<table>
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<th>Variable</th>
<th>Haloperidol</th>
<th>$^3$H-labeled ligands</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{\text{off}}$ (min$^{-1}$)</td>
<td>0.122</td>
<td>0.022</td>
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<tr>
<td>$k_{\text{on}}$ (min$^{-1}$ nM$^{-1}$)</td>
<td>0.085</td>
<td>0.086</td>
</tr>
<tr>
<td>$K_D$ (nM) calculated</td>
<td>1.44</td>
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</tr>
<tr>
<td>$K_D$ (nM) measured in vitro</td>
<td>1.3</td>
<td>0.10</td>
</tr>
<tr>
<td>$K'_I$ or $K_D$ (nM) measured in vivo (PET)</td>
<td>1.4</td>
<td>7.3</td>
</tr>
</tbody>
</table>

*Seeman, personal communication (human data).
*Köhler et al. (1985) (animal data).
*Wong et al. (1986b) ($K'_I$, human data).
*Farde et al. (1987b) ($K_D$, human data).
methylspiperone is sufficiently low. These issues are discussed below.

The least disputed classification of central dopamine receptors in the human distinguishes the two subtypes D₁ and D₂ on the basis of their ability to mediate activation of dopamine-stimulated adenylate cyclase (Spano et al. 1978; Kebabian and Calne 1979). Thus, the D₁ receptors mediate this activation, while the D₂ receptors inhibit it.

PET studies (Arnett et al. 1986) reveal significant D₂ sites outside the neuroendocrine areas only in the caudate-putamen regions of the basal ganglia. This finding coincides with the observation that significant dopa decarboxylase activity can be recorded in vivo with labeled dopa only in the neostriatum. Thus, quantitatively and in vivo, the caudate-putamen is by far the most important place of dopamine synthesis, synaptic release, and binding in the human brain.

Most dopamine receptor ligands bind to the D₂ sites with higher affinity than to the D₁ sites. The antagonists bind with equal affinity to the receptors in the low- and high-affinity states (Seeman 1985).

The binding sites of substituted benzamides (e.g., raclopride) have once been argued to be different from the sites labeled by other dopaminergic ligands, or to represent a subgroup of the D₂ sites (Creese et al. 1983). The evidence is neither conclusive nor compelling, however. It has also been suggested that the dopamine and butyrophenone (e.g., alkylspiperones) binding sites may be physically distinct because phenoxybenzamine destroys spiperone binding sites but leaves dopamine binding unaffected (Hamblin and Creese 1982).

A final point of considerable complexity is the location of the space from which the ligands gain access to the receptor sites. Dopamine does not passively cross biological membranes and hence can only bind to the receptors from the extracellular space. However, both methylspiperone and raclopride have sufficient intrinsic lipophilicity to cross the blood-brain barrier with first-pass extractions of 98–99 percent if they were not restrained by binding to plasma proteins. They may therefore also be able to cross presynaptic and postsynaptic membranes, and associate with binding sites that do not face the extracellular space. This also means that these ligands cannot be trapped in vesicles or inside membranes except by binding or metabolism. Chugani et al. (1987) presented evidence of agonist-induced endocytosis of spiperone binding sites in rat striatum in vitro, suggesting that bound spiperone remains attached to the binding sites during the internalization. This finding opens the possibility that some binding sites are nonfunctional in the sense that they do not face the synaptic cleft, as well as the possibility that changes of receptor turnover rates may affect methylspiperone and raclopride binding in other ways than by changing the number of receptors in the synaptic membrane.

**Methylspiperone.** Spiperone and its derivative alkyl-spiperone are among the ligands with the highest affinity for the dopamine D₂ receptor (Fields et al. 1977; see table 2). It binds with equally high affinity (0.1 nM) to the low- and high-affinity agonist-binding states of the D₂ receptor (Seeman 1985). It also binds to D₁ sites with low affinity (200 nM) and with considerable affinity (2 nM) to serotonin HT₂ sites, as well as to α₁-adrenergic sites in cerebral cortex (Hyttel et al. 1985). In addition, it may bind to muscarinic and histamine receptors but, of the secondary binding, only the binding to serotonin HT₂ sites is of quantitative interest to PET scanning.

The free fraction (f₁) of ¹⁸fluorine-labeled spiperone in human plasma is about 5 percent (Perlmutter et al. 1986). Protein binding is important because it affects the measurements of binding constants by inhibiting transfer of the ligand across the blood-brain barrier in both directions.

Determination of the free fraction in plasma used to determine f₁, the free fraction in brain, are usually performed in vitro and hence do not faithfully yield the “effective” free fraction in vivo under non-steady-state conditions. In addition, there is evidence that the protein binding in plasma is concentration dependent and can be blocked by prior treatment with unlabeled ligand because f₁ rose to 17 percent after blockade with cold ligand (Perlmutter et al. 1986). This finding, if confirmed, indicates that the nonspecific protein binding may be saturable.

There is no simple way of determining free fractions under non-steady-state conditions in vivo unless either the free fraction remains constant because of rapid equilibrium between nonspecifically bound and free, or the free quantity (not fraction) can be assumed to remain relatively more constant than the nonspecifically bound quantity (somewhat like the dissociation of oxygen from hemoglobin) because of rapid equilibrium between the free fractions in plasma and brain. Surprisingly, the f₁ of ¹⁸fluorine-labeled spiperone is even lower in brain, or only 0.5 percent
Metabolism of tracer spiperone in plasma and brain has been examined by several authors. In plasma, the study by Perlmutter et al. (1986) showed that measurable metabolites appear after a few minutes of circulation and that only negligible levels of unmetabolized spiperone exist after 3 hours. Arnett et al. (1985) measured 20 percent metabolites at 4 minutes and 80 percent at 4 hours. The ratio between metabolites and ligand was initially a practically linear function of the normalized plasma time-concentration integral, as expected from a simple first-order kinetic relationship between ligand and metabolites. This relationship was exploited in the methylspiperone metabolite correction introduced by Wong et al. (1986c). Significant tracer methylspiperone metabolites have not been found in brain (Arnett et al. 1985).

The most attractive pharmacological characteristic of methylspiperone is its enormous affinity which ensures high striatum-to-surrounding-tissue ratios. The disadvantage is the long studies required to reach equilibrium because of the very low rate of dissociation, and the consequent difficulty of obtaining equilibrium binding parameters. Equilibrium cannot be reached when methylspiperone is labeled with $^{11}$C. Thus, most of the PET studies using spiperone or its alkyl derivatives have been conducted in monkeys in order to extend the studies toward equilibrium. Only Wong et al. (1986b, 1986c, 1987) have, in fact, used spiperone or a spiperone derivative to derive $B_{\text{max}}$ values for dopamine D$_2$ sites in human neostriatum in vivo.

**Raclopride.** Raclopride belongs to a large group of substituted 6-methoxysalicylamides (benzamides) that pass into brain tissue in vivo in significant amounts. Its affinity for dopamine D$_2$ sites, as well as its lipid solubility, is not as great as that of spiperone or methylspiperone (table 2), indicating that it will reach equilibrium in a shorter period of time, as confirmed by PET. The substituted benzamides are the most selective D$_2$ antagonists known and thus do not label serotonin HT$_2$ sites in striatum or cortex.

The estimate of the $K_d$ of raclopride is several-fold higher in vivo than in vitro (7.1 nM; Farde et al. 1987b). This may suggest that not all of the ligand molecules in brain are free to associate with a receptor site. Since the apparent partition between plasma and brain is close to unity, it also suggests that the same percentage of free ligand exists in brain and plasma (i.e., about 4 percent; Köhler et al. 1985) but free fractions have not been measured in brain.

The metabolism of raclopride occurs in part by the formation of conjugates, including conjugates of raclopride itself. However, after 45 minutes, metabolites account for less than 20 percent of total radioactivity in human plasma (Farde et al. 1987a) and less than 10 percent in rat brain (Köhler et al. 1985) in vivo.

Raclopride has some practical advantages over methylspiperone as an in vivo tracer of dopamine D$_2$ receptor binding in the human brain. These include its high specificity for the D$_2$ site; its low affinity, which permits rapid equilibrium; and the relatively slow rate of metabolism. One difficulty of methylspiperone is the assumption of a value of $k'_{\text{on}}$ by which to calculate equilibrium binding constants, as well as the rapid metabolism in the circulation.

None of the pharmacological properties of methylspiperone explain the ability of methylspiperone to single out patients with psychoses who are known to be treatable with neuroleptics that block dopamine D$_2$ sites, regardless of whether they have in fact been treated or not. There remain, however, several unresolved potential differences of pharmacology between the two ligands that may affect the final judgment. It has not been definitively proved that methylspiperone and raclopride label the same sets or subsets of the D$_2$ sites, and it is not clear that the suggested process of receptor internalization must affect the two ligands in the same way. Given the current knowledge about these aspects of pharmacology, however, these concerns must at present be considered speculative.

**Modeling**

The issues addressed in this section concern the differences in the modeling approaches of the Karolinska and Johns Hopkins groups and the extent to which these differences may have produced the discrepancy in the results concerning drug-naive schizophrenic patients.

**Raclopride.** The analysis method was based on an equilibrium binding technique (Farde et al. 1985, 1986; Gjedde et al. 1986). Two measurements are made: one at high specific activity and one at a lower specific activity. For each study, a TAC is collected for the putamen and cerebellum. It is assumed that once equilibrium is reached, the cerebellum concentration reflects...
free ligand (F), and the putamen concentration equals bound (B) + free. The B/F ratio is plotted versus time to verify that equilibrium is reached. L. Farde reported that the B/F ratio is nearly constant from 36 minutes on. To reduce noise, the TACs for both cerebellum and putamen are fit to three exponentials and a concentration value is determined at 42 minutes postinjection. From these measurements and the measured specific activity of each injection, points for Scatchard analysis were derived. By plotting B/F versus B, B_max can be determined from the abscissa-intercept, and -1/K_d from the slope. Since only two measurements were in fact made, B_max and K_d were determined as the simple solution of two equations with two unknowns.

In the patient studies (Farde et al. 1987b), the low specific activity dose was designed to occupy approximately two-thirds of the receptors. This choice was made to minimize the variability introduced in measuring the small bound concentration as the difference between activities in putamen and cerebellum. L. Farde reported a correlation coefficient of 0.7 between B_max and K_d values and suggested that this correlation may be produced by common dependence of both measurements on the specific activity value. Errors in specific activity will move the low specific activity point on the Scatchard plot, resulting in a change of both intercept and slope. A systematic error in the calculation of specific activity resulted in the low values of B_max reported in the first study from this group (Farde et al. 1986). This error has been rectified in the subsequent publication (Farde et al. 1987b). In contrast, the different B_max values of the Hopkins control populations arise from the expansion of the normal volunteer population and the adoption of a fixed term for the values of the partition coefficient and k_off of haloperidol (D. Wong).

An essential assumption in the raclopride method is that the brain regions are at equilibrium 42 minutes postinjection. This is confirmed by plotting the B/F ratio versus time and verifying that it is not changing. The absolute concentrations in putamen and cerebellum, however, are changing over time due to clearance of tracer from the blood (figure 3 in Farde et al. 1985). Thus, there is only a secular equilibrium between plasma and brain. The half-life of plasma radioactivity was reported as 3-4 hours.

Another important assumption in the raclopride method is that the cerebellum concentration accurately reflects the concentration of free ligand. The Karolinska group has begun studies with the inert enantiomer of raclopride, 11C-FLB-472, in order to determine if the cerebellum is the most appropriate brain region to be used to estimate the free concentration in the putamen. An additional consideration is whether any of the "free" radioactivity is bound to nonspecific sites. L. Farde stated that approximately 96 percent of plasma raclopride is protein bound. However, the total radioactivity concentrations in cerebellum and plasma at equilibrium are comparable (Farde et al. 1985, figure 3). This would imply either that raclopride has a partition coefficient (i.e., the ratio between concentrations of free raclopride in plasma and brain at steady state) of 20-25 or, more likely, that a large fraction of raclopride in brain is not free. If there is significant nonspecific binding, the estimates of F from cerebellum would be overestimated, and B/F underestimated by a fixed percentage. It is likely that this would produce a 20- to 25-fold increase in slope (thus, a 20- to 25-fold reduction of K_d) with little or no change in B_max. However, the reported K_d values (7.1 nM) measured by PET are much larger than measured in vitro (1.2 nM).

Methylspiperone. The analysis method for N-methylspiperone (Wong et al. 1986a,b,c) is very different from the raclopride method. The use of a high-affinity ligand, 11C-N-methylspiperone, requires non-equilibrium kinetic analysis. The group used a three-compartment/four-parameter model to interpret the tissue TAC data observed (Gjedde et al. 1986; Gjedde and Wong 1987). One compartment represents N-methylspiperone in blood. The second compartment represents N-methylspiperone which is free, nonspecifically bound, or bound to rapidly equilibrating non-D_2 receptors in the striatum. The third compartment reflects specifically bound N-methylspiperone. The forward rate constant, k_s, expresses the rate of association of the ligand and receptor and is equal to the k_on B_max product. The Hopkins result hinges upon the observation of different values of k_s in schizophrenic and normal subjects. The cerebellar TAC is used to perform plasma metabolite corrections and to estimate tracer distribution volume by calculating the partition coefficient of 11C-N-methylspiperone. This distribution volume is used to correct for the binding of N-methylspiperone to non-D_2 receptors in the caudate (it has later been confirmed in vitro).
The parameter of interest is $k_3$, which is assumed to represent the product of the bimolecular association rate constant and the free receptor concentration. For the purpose of determination of $B_{\text{max}}$, two studies are performed per patient, both with high specific activity ligand. The first study is a control measurement, and the second is performed 4 hours after the administration of haloperidol. Two determinations of $k_3$ and a measurement of plasma haloperidol concentration (used to predict the tissue haloperidol concentration) are used to determine $B_{\text{max}}$ and $K_i$ from the slope and intercept of a straight line plot derived from two data points. Only two subjects have been studied more than twice to validate the linearity of this relationship. The method also assumes that the partition coefficient and dissociation rate ($k_{\text{off}}$) for haloperidol are constant and identical in the two groups. Like the Karolinska data, the Johns Hopkins data showed a correlation (0.7-0.8) between $B_{\text{max}}$ and $K_i$, which is due to the common dependency of both values on the measured data points.

The Johns Hopkins data demonstrated a significant change in $B_{\text{max}}$ between controls and patients and a large but not statistically significant change in $K_i$. The ratio of values of $k_3$ in the haloperidol-blocked and unblocked states is basically an index of saturation (85 percent in controls vs. 72 percent in drug-naive patients, but not significantly different). Because of the nonequilibrium nature of the method, it is inherently more complex than the methods used in the analysis of the raclopride data. There are, therefore, more assumptions used in the derivation of receptor parameters, and the effects of deviations from these assumptions on the measured values are also more complex. More studies to validate these assumptions would be useful.

In light of the previous discussion, the effect of differences in plasma protein binding between the control and patient populations was emphasized. A change in protein binding would produce a change in the apparent partition coefficient which would directly affect the estimated values of $B_{\text{max}}$ (and $K_i$). The change of the calculated value of $B_{\text{max}}$ would be proportional to variation of the ratio between free fractions in plasma and in brain. Thus, sufficient differences in protein binding between drug-naive and drug-treated patients may account for the apparent elevation of receptor concentration in drug-naive patients. At present, no studies have demonstrated significant differences in protein levels or free fractions between patients and normal subjects (Domino et al. 1975). Also, the resulting differences in protein binding of haloperidol or methylspiperone in vivo may be very difficult to measure directly.

**Summary, Conclusions, and Recommendations**

The power of PET studies to explore the neurochemistry of the human brain in vivo is enormous. Its applications are still in their infancy. Ultimately, these techniques have the power to map neuroreceptor systems in the normal human brain, to evaluate abnormalities in patients suffering from a variety of mental illnesses, and to explore relationships between clinical phenomenology, effects of treatment, and brain chemistry. In spite of the considerable promises and potential payoffs inherent in neuroreceptor imaging with PET, the workshop in Montreal also made it clear that the road to achieving these various goals will be long and arduous. It would be naive to expect two initial pioneering studies to solve fundamental questions concerning the pathophysiology of schizophrenia. The consensus of the outside observers was that the Karolinska and Hopkins teams showed admirable scientific integrity by submitting the details of their work for scrutiny and criticism. Continued dialogue of this type will certainly enhance our capability to extract maximal information from PET technology as quickly as possible.

The Montreal workshop made it clear that the development of radioligands and physiological models to measure neuroreceptors with PET is a very complex procedure. Many assumptions were required for the use of both N-methylspiperone and raclopride to determine $D_2$ density in schizophrenia. In each of the four areas examined (patient selection, scanning methods, pharmacology, and modeling), the two groups had different assumptions and different approaches. Their choices were clearly both defensible and reasonable, though different, given the current status of our knowledge. Ultimately, further validation studies will be required to verify the appropriateness of these assumptions and methods. A variety of detailed studies will be required to explore the effects of their different approaches on their results (e.g., differences in plasma protein binding between control and patient populations, differences in age, and chronicity of illness).
The workshop identified a series of differences between the Karolinska and Hopkins teams that could contribute to the divergent results. These include: (1) differences in age and chronicity in the two patient samples; (2) differences in age in the control groups; (3) differences in selection of the brain region to be examined (putamen in the Karolinska sample vs. caudate in the Hopkins sample); (4) differences in the metabolism of N-methylspiperone and haloperidol versus raclopride in the blood; (5) differences in the models, requiring an independently known value of $K_{on}$ in the Hopkins studies and equilibrium in the Karolinska studies; (6) possible differences between the binding sites labeled by N-methylspiperone and raclopride and the specificity of binding; (7) differences in PET instrumentation techniques; (8) possible effects of declining plasma protein binding on the steady-state volume of distribution of raclopride in the brain (i.e., the faster the plasma clearance, the higher the volume of distribution); (9) the possibility of a similar influence of this mechanism on the distribution of haloperidol in the Hopkins data; (10) possible differences in nutritional status that could affect plasma protein binding. The influence of these factors will only be determined by continued research.

But in isolation none appears to be able to account for the differences between subject groups in the same center, perhaps with the exception of differences of clinical characteristics. Acting additively, they may possibly explain the discrepancies observed.

The participants discussed possible recommendations for future studies at great length. They agreed that given the diversity of existing methodologies, the occurrence of discrepant results was not surprising and certainly not an occasion for pessimism. Answers to these and newer problems that will continue to arise will be solved through increasingly sophisticated use of PET methods. PET’s ability to map biochemical processes in the human brain is limited only by the further development of suitable radioligands, provided that the brain uptake of drugs and the selection of patients are recorded and analyzed with the same advanced skill that goes into ligand development and PET instrumentation.

The participants at the workshop reached a number of conclusions about suitable guidelines for future PET research and initial future directions. Clearly, one useful step would be for the two centers involved to apply the other’s methodology to the same patient sample. That is, the same patients and controls would be studied with both the equilibrium model using raclopride and the dynamic model using N-methylspiperone. While only a few groups are at present in the ideal position to conduct such studies on the basis of established experience in at least one of the existing methods, similar studies involving the use of both methods in the same patients may be useful in other centers as well. In studies which use pharmacologically diverse ligands in the same center (including both irreversibly and reversibly binding ligands), the patient populations must be evaluated with accurate medical and therapeutic histories and must be available for repeated scanning to permit longitudinal study of neuroreceptor function. Measurements of the development of symptoms with time and $D_2$ receptor density are essential to determine whether receptor up-regulation can occur as a concomitant of disease progression.

Other refinements in PET methodology will become increasingly important over time. For example, it will be important to determine in vivo protein binding in brain tissue and blood plasma; such estimates may be possible on the basis of TACs in blood and inert regions of the brain but will remain difficult given our ignorance of true in vivo equilibria. Additional increasing sophistication will also be required in measures of metabolite-plasma concentrations. Stabilization of head position and the development of improved techniques for anatomic definition are also important areas for future development. Ideally, regional measurements should be anatomically sophisticated and based on ‘objective’ maps generated through some standard established methodology such as CT, magnetic resonance imaging, or anatomically based computer atlases.

It was not possible to record the time course of accumulation of labeled drugs in brain in single subjects in vivo before the advent of PET. This unique capability of PET has also introduced kinetic problems that were unknown previously. Additional studies should, therefore, address the issue of dynamic sampling of radioactivity in all relevant compartments, including arterial blood. While this may not necessarily be required routinely in all studies, some basic research must be done involving arterial sampling to resolve these kinetic problems. TACs of the brain must be obtained with sufficient temporal resolution.
to record the approach to equilibrium faithfully.

It was the consensus of the workshop that our knowledge is not sufficient to recommend a single "best" technique for PET research involving neurotransmitters. This work remains in an early growth phase, and our knowledge during this phase is likely to advance if competent investigators are allowed maximal flexibility in exploring new areas and refining methodology in their particular areas of strength. Much additional work will be required to extract a maximum amount of useful information from the application of PET technology to the study of neurochemical systems in the human brain. Yet PET is the only existing in vivo technology suitable for this purpose. Since we assume that most mental illnesses, and particularly schizophrenia, are due to abnormalities in brain chemistry, PET will clearly be a powerful tool for psychiatric research during the next several decades.

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NARSAD (the National Alliance for Research on Schizophrenia and Depression) is a new collaborative effort of the National Alliance for the Mentally Ill, the Mental Health Association, the Schizophrenia Foundation, and the National Depressive and Manic Depressive Association. Working together, the citizens and professionals involved with NARSAD are marshaling support for research on psychiatric illness, primarily the schizophrenias and the affective disorders, but also—as the effort grows—other mental illnesses that cause major disability or dysfunction. The formation of this group coincides with the unprecedented promise offered by our current scientific armamentarium and reflects a burgeoning activism on the part of citizens' groups.

The primary goal of NARSAD will be to support the search for new information, new understanding, new ideas, and technological innovations that could shed light on the major mental illnesses. The ultimate goal is elimination of these illnesses through any and all efforts we can bring to bear. Many disciplines and approaches offer potential for better knowledge and improved methods of diagnosing, treating, and caring for our patients: genetics, biochemistry, pharmacology, neurobiology, epidemiology, psychiatry, psychology, physiology, and anatomy, among others. Within the framework of basic science, specific projects may have important implications for our biomedical, clinical, brain/behavioral, and therapeutic objectives—and such projects will be considered as well.

Grant applications for the first program of NARSAD have been reviewed, and the awards were made in April 1987. This program provides support for extension of research fellowship training. It is expected that as more resources become available, additional mechanisms will be supported. These may include faculty support, support for laboratory equipment, and other appropriate programs tailored to complement existing mechanisms available through the national institutes.

The Lieber Award, which was made in September 1987, is to further the research of an outstanding scientist carrying out work relevant to the causes, pathophysiology, treatment, and prevention of schizophrenia, depression, or other serious mental illness.

We are especially interested in fostering creative work and in minimizing the paper work and time usually expended responding to applications. We want to encourage young researchers entering the field or considering doing so, established scientists who have already been productive in these areas, and investigators using new or promising techniques that might be appropriate for psychiatric research or treatment. We are trying to use the knowledge and experience of our Scientific Advisory Board to avoid the problems known to accompany other support mechanisms.

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