Elevated D_2 Dopamine Receptors in Drug-Naïve Schizophrenics

D. F. Wong *et al.* (*1*) conclude that schizophrenia is associated with an increase in brain D_2 dopamine receptor density. This interpretation is based on the application of a mathematical model (*2, 3*) to data obtained with and without haloperidol pretreatment. However, alternative interpretations of the same data are possible. For example, as shown below, the data in table 1 of Wong *et al.* under the heading "$1/k_3$ before haloperidol" could be taken to indicate that schizophrenia may be associated with a decrease in brain D_2 dopamine receptor density.

The model that Wong *et al.* (*1*) apply is for (3-*N*-[^{11}C]methyl)spiperone ([^{11}C]-NMSP) accumulation in the caudate nucleus, as measured by positron emission tomography (PET). The central feature of the model is the effect of the nonradioactive inhibitor haloperidol on the kinetics for [^{11}C]NMSP accumulation. This effect is described by a parameter k_3 (*4*), defined as $k_{on}B'_{max}/V_d$, where k_{on} is the second-order molecular association constant for [^{11}C]NMSP binding to the receptor, B'_{max} is the density of receptors available for the binding of [^{11}C]NMSP, and V_d is the water volume of [^{11}C]NMSP in the brain [assumed to be numerically equal to 1.0 in (*3*)]. In the presence of a competitive inhibitor, the apparent k_3 is operationally defined by the relation

$$1/k_3 = [(K'_I + C_I)V_d]/[k_{on}B_{max}K'_I]$$

where K'_I is the apparent inhibitory constant for haloperidol with respect to the receptor, C_I is the brain concentration of haloperidol, and B_{max} is the total density of the receptor.

According to this relation, a plot of $1/k_3$ versus C_I will be linear, with an x-intercept equal to $-K'_I$, a y-intercept equal to $V_d/[k_{on}B_{max}]$, and a slope equal to $V_d/[k_{on}B_{max}K'_I]$. The two intercepts and the slope are not all independent, since any two of these parameters determine the third. It is assumed (*3*) that k_{on} is identical to the second-order rate constant k'_{on} for haloperidol binding to the receptor and that the value for the first-order rate constant k'_{off} for haloperidol dissociation from the receptor is accurately known. Thus the slope is expressed by $V_d/[k'_{off}B_{max}]$. This expression is used to quantify B_{max}.

The y-intercept, as described above, is equal to $V_d/[k_{on}B_{max}]$. Since V_d is assumed to be numerically equal to 1.0 (*3*), the y-intercept can be expressed as $1/[k_{on}B_{max}]$. The experimentally determined y-intercepts for each individual are shown in figure 3 of Wong *et al.* (*1*) ["Pre-haloperidol $1/k_3$" (*5*)], and the average values for normal individuals (11.7 ± 1.4 minutes) and for drug-naive schizophrenics (18.5 ± 2.4 minutes) are tabulated in table 1 of Wong *et al.* (*1*). According to the definition of the y-intercept, these values imply that $k_{on}B_{max}$ for the normal individuals is higher than $k_{on}B_{max}$ for the schizophrenics. This is inconsistent with the conclusion that schizophrenia is associated with an increase in brain D_2 dopamine receptor density (*1*), unless it is postulated that k_{on} for normal individuals is greater than k_{on} for schizophrenics.

In the absence of certain assumptions, the use of haloperidol provides no information about B_{max} beyond that which is available from the studies in the absence of haloperidol. On the other hand, interpretation of results in the presence of haloperidol is more complicated than in its absence, a number of assumptions are required, and incorrect conclusions might be drawn. The values for $1/k_3$ in the presence of haloperidol for each individual are shown in figure 3 of Wong *et al.* (*1*) ("Post-haloperidol $1/k_3$"). We computed the average values plus or minus the sample standard deviation for normal individuals (87.7 ± 22.9 minutes) and for drug-naïve schizophrenics (63.7 ±14.9 minutes). Because of the uncertainty (*4*) in the determination of $1/k_3$ in the presence of haloperidol, these means may not be significantly different from each other. Yet Wong *et al.*'s determination of B_{max} from the slope (*1*) indicates that schizophrenics have more than 2.5 times the receptor levels that normal individuals have. Thus there is an apparent inconsistency between the data and the conclusions (*1*) depending on whether the slope or the y-intercept is used.

In drawing the conclusion that schizophrenics have elevated receptor levels, Wong *et al.* (*1*) postulate that either K'_I for haloperidol is altered in the schizophrenics or that there is endogenous dopamine bound to the receptor in the schizophrenics. But an altered K'_I would contradict the basic assumption of the original model (*3*) that the value of k_{on} for [^{11}C]NMSP is identical to the value of k'_{on} for haloperidol, and that k'_{off} for haloperidol is a known constant value (*6*). Also, an altered K'_I does not explain the discrepancy between the value of B_{max} computed from the y-intercept as compared with the value computed from the slope.

Similarly, the postulation of endogenous dopamine contradicts the basic formulation of the original model [for example, equation 4 of Wong *et al.* (*3*)], where the mathematical derivation assumed no endogenous dopamine. This assumption has been emphasized by Wong *et al.* (*7*), who have said, "an effect of endogenous ligands is not likely with such high-affinity ligands as NMSP." In fact, Wong *et al.* (*8*) have shown that cocaine, a potent dopamine uptake inhibitor, had insignificant effect on in vivo NMSP binding in human caudate and cerebellum and have concluded that "endogenous dopamine release does not have a significant effect on NMSP binding in normal PET scan conditions." If endogenous dopamine were, in fact, an important factor, then the slope method used to quantify the PET data would be invalid, so that the conclusions of Wong *et al.* (*1*), which are based on this analysis, would be in error. A complicated analysis (*9*) would be required to take into account all the possible effects of the endogenous dopamine and of the added haloperidol (*10*).

In general, a number of alternative conclusions can be drawn whenever data containing significant experimental error (*4*) are analyzed and plausible, but arbitrary, postulations are applied. In the case discussed here, without introducing an inconsistency in the interpretation of the x-intercepts, y-intercepts, or slopes, a different set of assumptions might lead to the conclusion that schizophrenics do not have elevated receptor levels.

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REFERENCES AND NOTES

1. D. F. Wong *et al.*, *Science* **234**, 1558 (1986).
2. D. F. Wong, A. Gjedde, H. N. Wagner, Jr., *J. Cereb. Blood Flow Metab.* **6**, 137 (1986).
3. D. F. Wong *et al.*, *ibid.*, p. 147.
4. Since the k_3 values used for the analysis were derived from a model (*2, 3*) that accounted for the effects of blood flow and of [^{11}C]NMSP delivery, the k_3 values should theoretically reflect only the single step of [^{11}C]NMSP binding to the receptor. In practice, however, $1/k_3$ determined in the presence of haloperidol is subject to uncertainty, since most of the receptor is unavailable for [^{11}C]NMSP binding and only small amounts of receptor-ligand complex are formed. Two effects of this are that the signal

from the complex will be small and will lead to large statistical (radioactivity counting) errors and that the signal from the unbound or nonspecifically bound NMSP in the brain may interfere with the signal from the complex. The $1/k_3$ value derived in the presence of haloperidol is the primary determinant of the slope [figure 4 of (*3*)] used to quantify B_{max}. Values for $1/k_3$ determined in the absence of haloperidol may be inaccurate because, for high levels of available receptor, the kinetics of [^{11}C]NMSP accumulation in the caudate nucleus primarily reflect blood-brain transport and are relatively insensitive to the second-order binding step k_{on}, so there may be significant errors associated with the numerical extraction of k_3 from the observed data. Values for $1/k_3$ derived in the absence of haloperidol would be the primary determinant of the *y*-intercept [figure 4 of (*3*)]. In the absence of additional information about the analysis of the original experimental data or a sensitivity analysis based on computer simulation studies, it is not clear what the magnitude of these errors might be.

5. The *y*-intercept and $1/k_3$ before haloperidol treatment will be identical, since $1/k_3$ before haloperidol treatment is one of the two points used in the $1/k_3$ versus haloperidol plot.
6. Since K'_1, equal to k'_{off}/k'_{on} and determined from the *x*-intercept of the $1/k_3$ versus haloperidol plot, is different for normal individuals and for schizophrenics (*1*), k'_{off} or k'_{on}, or both, must be different for normal individuals and for schizophrenics. If k'_{off} differs, then in the implementation of the slope method a single assumed constant value cannot be used for both normal individuals and for schizophrenics. If k'_{on} differs, one would need to assume that k_{on} also differs in such a way that k_{on} (normal)/k_{on} (schizophrenic) is identical to k'_{on} normal/k'_{on} (schizophrenic).
7. D. F. Wong *et al.*, *Science* **232**, 1270 (1986).
8. D. F. Wong *et al.*, *J. Nucl. Med.* **27**, 1074 (1986).
9. P. Seeman, *Pharmacol. Rev.* **32**, 229 (1981); A. A. Hancock and C. L. Marsh, *Mol. Pharmacol.* **26**, 439 (1984).
10. In addition to the direct result of haloperidol and dopamine competition in [^{11}C]NMSP binding to the D_2 receptor, an analysis of the effects of endogenous dopamine and added haloperidol must take into account the indirect result of the influence of haloperidol on the turnover of dopamine, on occupancy, functioning, and regulation of the presynaptic dopamine autoreceptor and the postsynaptic D_1, D_2, and D_3 dopamine sites, and on the levels of [^{11}C]NMSP and dopamine in the synaptic cleft. For example, D. C. Chugani *et al.* [*J. Nucl. Med.* **28**, 612 (1987)] have shown that endogenous dopamine can increase in vivo ^{3}H-spiperone binding by stimulation of endocytotic trapping.

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Response: We appreciate the comments of Zeeberg *et al.*, which give us an opportunity to amplify our conclusions. On the basis of simple receptor kinetic theory, we predicted that the reciprocal of the binding coefficient ($1/k_3$) must be a linear function of the inhibitor (haloperidol) concentration (*1*). The plot of $1/k_3$ versus haloperidol concentration is essentially a Woolf plot (*2*) in which the slope equals the value of $1/[k'_{off} B_{max}]$, where k'_{off} is the in vivo rate of dissociation of haloperidol from the receptor sites, corrected for the ratio between the k_{on} values of (3-*N*-[^{11}C]methyl)spiperone ([^{11}C]NMSP) and haloperidol. Zeeberg *et al.* assert that it may be equally valid to calculate the value of B_{max} as the ratio between k_3 and k_{on} in the absence of any inhibitor and subsequently use the published values of the ordinate intercept to suggest a contradiction in our conclusion that B_{max} is higher in drug-naïve schizophrenic patients. The data on the ordinate intercept and affinity were cautiously presented in our report so that future studies might shed light on issues such as possible elevated neurotransmitter levels in schizophrenics, while the principal point of the report concerned receptor densities. We believe the empirical differences and the theoretical arguments that lead to the assertions of Zeeburg *et al.* are not robust. In fact, the observed values of the ordinate intercept in patients and controls are quite compatible with our original thesis.

The argument extended by Zeeburg *et al.* is drawn solely from comparisons between patient and control groups for the ordinate intercept values. However, as we stated (*3*), these differences were not statistically significant. Basing the calculation of B_{max} on the assumed value of k_{on}, rather than on the assumed value of k'_{off}, did not work in our experience for several reasons. First, we did not know the in vivo value of k_{on} for [^{11}C]NMSP, while an estimate of k'_{off} could be obtained from the literature. Second, estimates of k_3 in the absence of inhibition are more uncertain than in the presence of inhibitor because binding to unblocked receptor sites is sometimes so intense that delivery of tracer from the circulation to tissue may affect the accuracy of the binding estimates. Measurement of cerebral blood flow will not facilitate the calculation of the binding rate in this situation, but will merely confirm that binding has little influence on the rate of tracer accumulation. Third, in our case with the use of Woolf plot, the theoretical and experimental accuracy of B_{max}, determined as the reciprocal value of a slope, has a much lower relative variance than the K_D estimated from the ordinate intercept (*2, 3*). Fourth, the solution of the equation for B_{max} incorporates k_3 both in the presence and in the absence of haloperidol. The $1/k_3$ averages near the origin of the graph have less influence on the calculated B_{max} values for relatively sizable haloperidol concentrations because the k_3 value in the presence of haloperidol dominates the calculation. In our data $1/k_3$ observed in the absence of haloperidol is on average only 25% (0 to 60%) of the value of $1/k_3$ obtained in its presence.

Even if the ordinate intercept values were significantly different between patients and controls, they would merely imply a greater increase of the observed k_{on} for control subjects than for drug-naïve schizophrenic patients. An increased total number of dopamine receptors in schizophrenics may accompany a decreased rate of association, which is reflected in our report of a higher K'_1 value for haloperidol. Reduced affinity is a common consequence of up-regulation of receptors, perhaps due to impaired access to the receptor sites in vivo or large increases in endogenous neurotransmitter competition. The ratio between the rates of net binding of methylspiperone in the haloperidol-blocked and unblocked cases is a model-independent estimate of the in vivo affinity of haloperidol.

An isolated increase of this value of K'_1 in drug-naïve schizophrenics would be difficult to explain, save by a decrease of the in vivo value of k_{on}. In fact, we stated that an increase in K'_1 (and logically k_{on}) could be predicted and be consistent with our analysis and findings.

Zeeberg *et al.* argue that our previous reports indicate a lack of endogenous competition with NMSP binding to receptors. The studies of cocaine administration to young subjects used a different modeling approach, the so-called caudate/cerebellar ratio method, which may reflect both flow and receptor binding (reference 19 of our report). Given our current kinetic approach, the lack of change in the caudate/cerebellar ratio in the presence of intravenous cocaine does not exclude a reduction in the rate of binding of [^{11}C]NMSP due to endogenous competition (*4*).

An initial assumption of setting the endogenous effect to zero ($B_{max} = B'_{max}$, in the unblocked case) would be reflected in an increase in k_{on} if such competition should occur. Alternatively, an increase of k_{off} to explain the increase K'_1 would counter the argument advanced by Zeeberg *et al.* In this case we are left with the conclusion drawn in our original report, that is, that receptor numbers are elevated in drug-naïve schizophrenics. We showed an elevation in drug-naïve as well as in previously treated patients, in whom increased receptors have frequently been confirmed post-mortem. The criticism of Zeeberg *et al.* would equally affect this latter group of patients, if valid.

Zeeberg *et al.* also make comments regarding computations which may benefit from our clarification and response. They compare $1/k_3$ averages in the haloperidol-blocked cases. However, this comparison has meaning only when the haloperidol levels in blood are the same in normals and schizophrenics. When Zeeberg *et al.* averaged the $1/k_3$ values from figure 3, they apparently included values obtained at different haloperidol levels and thus did not correctly represent the "slope" differences. All haloperidol levels and corresponding $1/k_3$ values are shown in figure 3; B_{max} differences were dependent on the "slopes," but not on either $1/k_3$ or haloperidol alone. Zeeberg *et al.* also find it surprising to