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COMMENTARY

The thought-provoking article by Seeman et al. has three main components. The first component summarizes the evidence that the apparent affinity of D_2-like receptor antagonists varies linearly with the tissue–buffer partition coefficient of the radioligand and proposes that a radioligand-independent affinity value can be obtained by extrapolating from the linear relationship to estimate the equilibrium dissociation constant at the zero partition intercept. The second component validates the radioligand-independent dissociation constant by using values for several antipsychotic drugs to compare calculated therapeutic concentrations of the drugs with empirically determined in vitro concentrations. The third component then uses the radioligand-independent dissociation constants for antipsychotic drugs to examine several hypotheses for the atypical action of some antipsychotic drugs.

The observation that the apparent affinity of D_2-like receptors for many antagonists varies in a radioligand-dependent manner is important and provides a partial explanation for the wide range of affinity values reported in the literature for many of these ligands. Furthermore, the date demonstrate fairly convincingly that, for the D_2-like D_2 and D_4 receptors, the relationship between the observed equilibrium dissociation constant and the tissue–buffer partition coefficient is linear. Finally, extrapolation of this linear relationship to zero partition produces radioligand-independent affinity values for three drugs, chlorpromazine, haloperidol, and clozapine, that agree closely with K_D values obtained by saturation analysis of the binding of the corresponding [^{3}H]-ligands to the D_2 receptor.

As persuasive as these findings are, there are several ways in which the concept of a radioligand-independent dissociation constant could be strengthened. First and foremost, the concept would be more readily acceptable if a mechanism for the phenomenon of the dependence of the dissociation constant on the tissue–buffer partition coefficient of the radioligand were identified. For a simple competitive interaction, the apparent dissociation constant of a drug determined indirectly by its ability to inhibit radioligand binding ought to be equal to its dissociation constant determined in a direct binding assay. Several explanations for the discrepancy that are based on experimental artifacts have been excluded, which suggests that the interactions are not strictly competitive, and that the extent of the deviation from the results predicted by a model of competitive inhibition depends on the lipophilicity of the radioligand.

Second, a more precise definition of the tissue-buffer partition coefficient would be advisable. Seeman and Van Tol (1995) obtained this coefficient from the amount of radioligand nonspecifically bound at a 1 nM concentration of the radioligand, a determination that would appear to have considerable possibility for error because of the binding of the radioligand to the filter, variations in protocols for rinsing filters prior to scintillation counting, or specific (but not displaced) binding to a receptor subtype that has a low affinity for the ligand used to define nonspecific binding.

Third, there are a few minor inconsistencies and gaps in the data supporting the concept of the radioligand-independent dissociation constant. For example, K_D values for [^{3}H]-ligands are assumed to represent binding at close to zero partition, because the [^{3}H]-ligand does not compete with other compounds for binding the receptor, yet the K_D values for the binding of [^{3}H]-nemanopride, [^{3}H]-spiperone, and [^{3}H]-raclopride to the D_2 receptor, like drug K_I values, decrease when the incubation volume is increased from 1.5 to 8 ml (Seeman and Van Tol 1995), and the zero partition value for raclopride (0.6 nM) is 2- to 3-fold lower than its K_D value of 1-2 nM (Fig. 2b). It is also important that the same receptor preparation be used in the small and large volume experiments, whereas in Seeman and Van Tol (1995) the small volume experiments were carried out using $D2_L$, where $D2_S$ was used in the large volume experiments. Furthermore, although the results of Durcan et al. (1995) are in qualitative agreement with the date shown by Seeman et al. and demonstrate that the apparent affinity of the D_2 receptor for several antagonists varies according to the radioligand, plotting the data of Durcan et al. as depicted in Figure 2 of the review suggests very different values for the radioligand-independent dissociation constant. For clozapine, for example, the zero partition intercept would be approximately 7 to 8 nM, substantially less than the radioligand-independent dissociation constant of 44 nM calculated by Seeman et al. (Table 1 of the review).

As additional validation of the concept of radioligand-independent dissociation constants, Seeman et al. demonstrate that calculated therapeutic concentrations derived from these constants closely predict observed plasma

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water concentrations of a number of antipsychotic drugs. Although the close correspondence between calculated and observed concentrations is impressive, the conclusions do not appear to take into account the substantial between-patient variability observed for plasma concentrations of antipsychotic drugs, and multiple assumptions are required for the calculations. To calculate in vivo receptor occupancy in the presence of endogenous dopamine, it is necessary to know both the concentration of dopamine and its affinity for the receptor, in addition to the extrapolated radioligand-independent dissociation constant. Although Kawagoe et al. (1992) estimated that the basal *extrasynaptic* concentration of dopamine in the striatum of the anesthetized rat is less than 6 nM, they determined that stimulation may produce transient concentrations almost 100-fold higher, and Wightman and colleagues have calculated that in the rat nucleus accumbens the stimulated *synaptic* dopamine concentrations may be transiently as high as 1.6 mM (Garris et al. 1994). I would argue that we have insufficient data to make an accurate estimate of the synaptic concentration of dopamine in freely moving patients chronically treated with antipsychotic drugs, but that the concentration is likely to be much higher than 10 nM. Similarly, the affinity of D_2-like receptors for dopamine is assumed to be equal to the high-affinity dissociation constant for dopamine determined in an membrane preparation, whereas it might be more reasonable to determine a functional K_A in an intact cell system. One such analysis for the D_2 receptor suggests an affinity for dopamine of about 0.6 μM (Mak et al. 1995), a value considerably higher than is typically obtained for high-affinity binding of dopamine to membranes.

The major aim of the review by Seeman et al. is to use the radioligand-independent dissociation constants of antipsychotic drugs at D_2, D_4, and 5-HT_{2A} receptors to evaluate hypotheses for the atypical actions of some of the drugs. The conclusions rest, therefore, on the validity of the radioligand-independent dissociation constant, which, as discussed, seems reasonably well established for D_2 and D_4 receptors. It also is important to keep in mind that antipsychotic drugs can be classified as atypical on the basis of their reduced ability to induce extrapyramidal side effects such as Parkinsonism, or on the basis of an ability to treat neuroleptic nonresponders and symptoms not normally alleviated by typical neuroleptics. The latter basis for classification as atypical, clinical efficacy, can be evaluated only in humans, and the one antipsychotic drug that is generally considered to be atypical in this respect is clozapine (Deutch 1995, Kane et al. 1988), whereas other atypical antipsychotic drugs belong to the first class. Thus, until more drugs that treat negative symptoms or neuroleptic nonresponders are identified, any attempt to correlate receptor binding affinities with behavioral responses can only address the first definition of atypicality, a reduced likelihood of inducing catalepsy or Parkinsonism, which is the definition put forth in the review.

The first distinction between atypical and typical antipsychotic drugs noted by Seeman et al. is that the radioligand-independent dissociation constants for some atypical drugs are higher (lower affinity for the D_2 receptor) than they are for the typical neuroleptics. The authors propose that the lower affinity of the atypical antipsychotic drugs renders them more susceptible to displacement by endogenous dopamine. Why should this be the case, even though the lower affinity compounds are present at higher concentrations, and displacement by dopamine should depend on the ratio of the drug concentration to its affinity, rather than on only the affinity of the drug? The answer may lie in the likelihood that dopamine is not at equilibrium with its receptors in vivo. It may be that during a pulse of dopamine release in the synapse, the more rapid dissociation of a lower affinity antagonist increases the probability that dopamine will bind to the receptor and prevent rebinding of the antagonist, whereas there is a lower probability that a given receptor occupied by a higher affinity (more slowly dissociating) antagonist will become unoccupied during a dopamine transient. However, if lower affinity, and thus lower receptor occupancy, were sufficient to produce an atypical drug profile, simply reducing the dose of haloperidol should improve its clinical profile, as noted elsewhere (Kapur 1996). The final part of this hypothesis is that regional dopamine concentrations must differ, so that atypical antipsychotic drugs are preferentially displaced by high dopamine concentrations in brain regions responsible for the extrapyramidal side effects of D_2 receptor blockade, whereas low concentrations of dopamine in brain regions responsible for the therapeutic actions of the drugs permit the binding of both typical and atypical antipsychotics. Although the concentrations of dopamine and dopamine metabolites are higher in the striatum than in other nuclei of the basal ganglia such as the nucleus accumbens, the relative concentration of dopamine *at D_2 receptors* will depend on many factors such as whether the receptors are synaptic or extrasynaptic, the amount of dopamine released per terminal, the density of autoreceptors, and the density and activity of dopamine transporters per terminal. Furthermore, as antipsychotic drugs can alter dopamine release in a brain region- and drug-dependent manner, it is clear that additional work is needed to establish more firmly the relative concentrations of dopamine at D_2 receptors in various brain regions of drug-treated patients.

For clozapine and for those atypical antipsychotic drugs with high affinity for D_2 receptors, Seeman et al. propose that D_4 receptor selectivity is the basis for their

atypical actions. To support their claim, they demonstrate the existence of a remarkable sigmoidal relationship between the dose of antagonist required to produce catalepsy in rats and the ratio of radioligand-independent dissociation constants at D_4 and D_2 receptors. As noted, this relationship rests on the validity of the calculated affinity values. Much more work is needed to demonstrate that a correlation does not exist between 5-HT_{2A} receptor selectivity and atypical antipsychotic action. It is difficult to place much confidence in the radioligand-independent dissociation constants for the antagonists of 5-HT_{2A} receptors, because the values are based on linear regression with, and extrapolation from, only two data points (Figure 2 in the review). For other hypothetical mechanisms of atypicality based on the receptor selectivity profile, such as hypotheses involving anticholinergic or antiserotonergic actions, it has been difficult to demonstrate that the combined use of two specific drugs has the same effect as the use of one drug with activity at both receptors (Deutch 1995; Kapur 1996). As new D_4-selective compounds are developed, I look forward to seeing them tested in this paradigm.

This review presents data systematically characterizing an important phenomenon, the dependence of the apparent affinity of dopamine antagonists on the radioligand used in competition bindings assays. The results suggest the testable hypotheses that low affinity for the D_2 receptor and D_4 receptor selectivity contribute to the reduced likelihood of eliciting Parkinsonism associated with atypical antipsychotic drugs. Interestingly, clozapine belongs to both the low-affinity class and the D_4-selective class of atypical drugs. Seeman et al. argue persuasively that susceptibility to displacement by dopamine and D_4 selectivity both warrant consideration as mechanisms of atypical action.

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