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## COMMENTS ON A COMMENTARY

Commentary articles are written to explore provocative new ideas and developing or controversial areas of research. To increase the exchange of information and ideas, we are publishing the following *Comments on a Commentary*, in which Dr. Ian Creese and Dr. David R. Sibley offer their observations on the article "Nomenclature of Central and Peripheral Dopaminergic Sites and Receptors" by Dr. Philip Seeman, published in this issue, and also provide some alternative thoughts on this important area of research. From time to time in the future we will solicit similar constructive comments on selected *Commentary* articles from experts in appropriate fields.

While the final decision on selection of authors for either *Commentaries* or *Comments on a Commentary* will be made at the discretion of the editors, we welcome suggestions from readers, which should be addressed to: Dr. C. N. Gillis, Department of Pharmacology, Yale University School of Medicine, 333 Cedar St., New Haven, CT 06510, U.S.A., or Professor Z. M. Bacq, Laboratoire de Pathologie et Therapeutiques, Université de Liège, 32 Boulevard de la Constitution, Liège, Belgium.

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# COMMENTS ON THE COMMENTARY BY DR. SEEMAN

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Seeman's attempt to equate radioligand binding data [1] with more customary approaches to classify dopamine receptors is to be commended. Because radioligand binding measures receptor occupancy directly and the pharmacological effects of antagonists are proposed to be mediated solely through receptor occupation, a direct comparison of their molar potencies is theoretically correct. However, although the *rank order* potencies of agonists may be identical in such comparisons, their *absolute* molar potencies need not agree. A physiological response may not be linearly related to agonist binding because of spare receptors, efficacy considerations, high efficiency receptor/effector coupling mechanisms or intracellular amplification events. These caveats have not been adequately considered to warrant the proposed classification.

We disagree with the conclusion that $D_2$ and $D_4$ sites represent distinct dopamine receptors. It should be noted that antagonists have identical affinities at both sites. The distinguishing feature between $D_2$ and $D_4$ sites is that of absolute molar agonist affinities. Seeman has made a number of errors in his interpretation of these data. The first problem lies in his derivation of agonist potencies for $D_2$ sites. He uses $IC_{50}$ values from agonist/$^3$H-antagonist displacement curves in membrane preparations. Such curves have low Hill coefficients (<1) and are

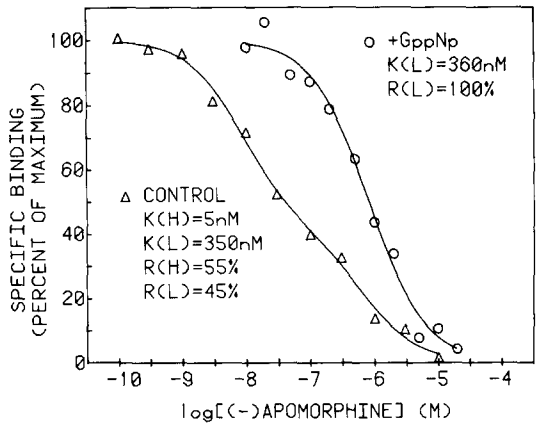


Fig. 1. Computer modeled curves for a (−)apomorphine/[$^3$H]spiroperidol competition experiment in bovine anterior pituitary membranes.

biphasic, possessing more than one binding component. Such $IC_{50}$ values are biologically meaningless. We have made a detailed computer analysis of agonist/$^3$H-antagonist displacement curves to D-2 receptors in the pituitary [2], and it is clear that one can identify distinct high- and low-affinity binding components. We have designated the high- and low-affinity components $R_H$ and $R_L$ with the respective dissociation constants $K_H$ and $K_L$ (Fig. 1). These agonist-defined high- and low-affinity components would thus correspond to Seeman's $D_4$ and $D_2$ sites. However, our conclusion is that these components are two states of a *singular D-2 receptor*. This is supported by the finding that in the presence of guanine nucleotides only the low-affinity component is observable (Fig. 1). Since nucleotides do not affect $^3$H-antagonist binding, which measures $R_H$ plus $R_L$, then there must be conversion of $R_H$ to $R_L$.

Seeman does not present data (nor are any available) for *molar* agonist potencies representative of physiologic $D_2$ *site* interactions. The statement that "$IC_{50}$ values of agonists . . . at this site correlate very well with the doses which elicit the various dopaminergic behaviors" is misleading since such correlations are based on molar $IC_{50}$ values of agonists in displacing $^3$H-antagonist binding with mg/kg of peripherally administered agonists which induce central behavioral effects.

A second problem arises when Seeman compares his $D_2$ agonist $IC_{50}$ values in membrane binding experiments with pharmacologically derived $EC_{50}$ values, using intact tissues. Based on the observation that the former values are higher than the latter ones, he concludes that different receptor subtypes ($D_2$ and $D_4$) are involved. For reasons discussed above, however, this comparison is error prone and is not a sufficient criterion for receptor classification. With this caveat in mind, one should note that there is a good correlation between the $D_2$ $K_H$ values for agonists and their $EC_{50}$ values in producing D-2 responses. Since $^3$H-agonists only label the $R_H$ state of the D-2 receptor, agonist/$^3$H-agonist displacement curve affinities represent these $K_H$ values as seen in Table 6. [1]. Of course, absolute affinities of agonists are highly dependent on assay conditions. For instance, the $K_H$ for dopamine can vary between 5 and 300 nM depending upon the cation concentration and the temperature of the assay. Furthermore, comparisons with absolute potencies in hormone release experiments are suspect because dopamine agonists are unstable and incubations are conducted for up to 2 hr.

We agree with Seeman's categorization of D-1 receptors. However, receptors linked to stimulation of adenylate cyclase also demonstrate a high-affinity agonist binding state in membranes. Thus, some $D_3$ binding of $^3$H-agonists may represent labeling of this state of the D-1 receptor. One should note that antagonist affinities at D-1 and $D_3$ sites are similar, and some high-affinity agonist (low-affinity antagonist) binding sites are GTP sensitive which supports this hypothesis. In addition, the statement that the rank order of potencies of drugs at the $D_2$ site is the same as that for the D-1 site is incorrect. At D-1 sites phenothiazines are more potent than butyrophenones whereas at D-2 sites butyrophenones and phenothiazines demonstrate high affinity.

Finally, although Seeman is to be commended for attempting to integrate studies of peripheral dopamine receptors with those in the CNS, many of these studies have been conducted under conditions where tissue has been pretreated with phenoxybenzamine, an agent which is now known to irreversibly inactivate some classes of dopamine receptors [3]. Furthermore, in dealing with intact tissue it is unclear whether some of the pharmacological effects observed might be mediated through interactions with other neurotransmitter receptors present in the tissue.

In conclusion, our categorization of dopamine receptors is as follows:

D-1 receptors: Dopamine receptors associated with stimulation of adenylate cyclase activity, labeled by $^3$H-thioxanthenes and possibly by $^3$H-agonists. Low affinity for butyrophenones and sulpiride.

D-2 receptors: Dopamine receptors associated with inhibition of adenylate cyclase activity labeled with high affinity by $^3$H-antagonists. $^3$H-Agonists label a high-affinity state of this receptor in the absence of guanine nucleotides. Guanine nucleotides convert this high-affinity agonist binding state to a low-affinity agonist binding state.

D-3 binding sites: Sites labeled with high affinity by $^3$H-agonists which demonstrate low affinity for butyrophenones. Since no physiological effect has been associated directly with these sites, they may not, as yet, be termed receptors.

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