MOLECULAR PHARMACOLOGY, 21:315–319

Differential Stereoselectivity of Methotrimeprazine Enantiomers for Selected Central Nervous System Receptor Types

T. A. ROBERT, A. N. HAGARDORN, AND E. A. DAIGNEAULT

Department of Pharmacology, Quillen-Dishner College of Medicine, East Tennessee State University, Johnson City, Tennessee 37614

Received June 22, 1981; Accepted November 24, 1981

SUMMARY

Optical isomers of methotrimeprazine, an analgesic/neuroleptic, were investigated with respect to their ability to interact with six receptor types or subtypes. Bovine caudate nucleus tissue homogenates provided the dopamine, opiate, and serotonin receptor populations studied in these experiments. The radioligands used in saturation and binding competition experiments were tritiated dopamine, spiperone, dihydromorphine, 5-L-methionine enkephalin, naloxone, and 5-hydroxytryptamine. Saturation experiments verified acceptable performance of these *in vitro* receptor assay systems and indicated that a one-site binding model was adequate for each of these ligands under the experimental conditions employed. The competition experiments exhibited statistically significant ($p < 0.05$) differences in isomeric effects only for dopamine and 5-hydroxytryptamine receptors. The more active isomer, levorotatory methotrimeprazine, was shown to be pharmacodynamically equivalent to chlorpromazine at these receptor types. When the magnitude of receptor stereoselectivity is plotted against an estimate of the more active isomer's affinity for that particular receptor, an excellent correlation is observed. This suggests that a high degree of stereoselectivity characterizes a highly specific drug/receptor interaction. These findings are compatible with the conclusion that methotrimeprazine does not produce analgesia via a direct action upon opiate receptors.

INTRODUCTION

Optical isomer pairs exhibit unique physicochemical properties which permit them to serve an important role in pharmacological investigations. Enantiomers possess identical physical properties and exhibit differences only at the molecular level, where they interact with selective systems which can discriminate between their particular stereochemical orientations. Receptor stereoselectivity has become an operational criterion of receptor identification (1). Further information may be obtained from considerations of drug/receptor stereoselectivity as was first proposed by Pfeiffer in 1956 (2). This postulate predicts that, with greater potencies of drugs, larger differences in pharmacological effects will be seen between the enantiomers. The converse of this proposition is that the greater the difference between receptor affinities of optical isomers, the greater will be the pharmacological effect of the active specie. This postulate was also presented by Pfieffer (2) in specific reference to the differential tissue responses (via receptor subtypes) to optical isomers of sympathomimetic amines.

We have undertaken a series of experiments based upon a corollary of Pfieffer's theory: the greater the difference in pharmacological activity of enantiomers, the more specific is the effect of the active isomer at a particular receptor type in a particular tissue. We have attempted to utilize the intrinsic stereoselectivity of several receptor types in a single region of bovine brain to characterize various pharmacological activities of MTM[1] enantiomers. As a member of the vast family of phenothiazine derivatives, MTM possesses a typical impressive array of pharmacological activities (3) which tends to confound analysis of its relevant therapeutic actions. Unlike the majority of aliphatic phenothiazine derivatives, the chiral center in MTM engenders the potential for enantiomeric stereoselectivity analysis. Unlike the dextrorotatory enantiomer, (+)-MTM, the levorotatory isomer, (−)-MTM, occupies a singular position among phenothiazine derivatives in that it possesses clinically useful analgesic effects. The drug is also used as a neuroleptic in Europe. The mechanism of (−)-MTM analgesia has remained obscure (4), whereas its antipsychotic effects may be attributable to antidopaminergic activity identified in other phenothiazine neuroleptics via *in vitro* radioligand binding studies (5).

Specific central nervous system receptor types were

This work was supported in part by Biomedical Research Development Grant 1-S08-RR-09171-03.

[1] The abbreviations used are: MTM, methotrimeprazine; DA, dopamine (dihydroxyphenylethylamine); SP, spiperone; DHM, dihydromorphine; ENK, enkephalin; NAL, naloxone; 5-HT, serotonin (5-hydroxytryptamine); CPZ, chlorpromazine; ANOVA, analysis of variance.

0026-895X/82/020315-05$02.00/0

compared in their ability to interact with MTM enantiomers in order to estimate the relative significance of each such interaction. Dopamine (DA and SP), opiate (DHM, ENK, and NAL), and 5-HT receptors or receptor subtypes in calf caudate nucleus were examined for their relative abilities to discriminate between (+)- and (−)-MTM. It is believed that data of this type will provide a useful basis for quantitative assessment of pharmacological effects. We report here our findings regarding the stereoselectivity profile of MTM isomers at these three receptor types. An additional comparison was made between the effects of (−)-MTM and the prototypical aliphatic phenothiazine derivative CPZ at dopamine and 5-HT receptors.

MATERIALS AND METHODS

Tritiated receptor ligands were obtained from New England Nuclear Corporation (Boston, Mass.) (dihydroxyphenylethylamine, [^{3}H]DA, specific activity 15.4 Ci/mmole; [^{3}H]NAL, specific activity 50.0 Ci/mmole; 5-hydroxytryptamine creatinine sulfate, [^{3}H]5-HT, specific activity 28.9 Ci/mmole; 5-L-methionine enkephalin, [^{3}H]ENK, specific activity 25.8 Ci/mmole) or Amersham (Arlington Heights, Ill.) ([^{3}H]SP, specific activity 20 Ci/mmole; [^{3}H]DHM, specific activity 70 Ci/mmole). All general reagents used in the radioligand binding assays were purchased from commercial suppliers in American Chemical Society reagent grade. Pargyline, bacitracin, CPZ, and Tris buffers were purchaed from Sigma Chemical Company (St. Louis, Mo.). Isomers of MTM-HCl were the gift of Rhone Poulenc (Paris, France; (+)-butaclamol-HCl from Ayerst Laboratories (Montreal, Que.); methysergide maleate was a gift and levalorphan tartrate was purchased from Roche (Nutley, N. J.). Fresh (less than 1 hr after slaughter) calf brain tissue (anterior caudate nucleus) was the generous gift of Valleydale Packers Inc. (Bristol, Va.).

Radioreceptor assays were performed following well-documented techniques from the literature. Specifics of the various methods are outlined in order to emphasize those aspects which were either subject to alteration from the original references or to illustrate uniformities between our experiments. All radioreceptor assays with a particular tritiated ligand and a single batch of tissue homogenate were performed in two discrete parts. A saturation assay was performed to verify the performance of each *in vitro* determination by subsequent Scatchard analysis and comparison with known K_D and B_{max} values. Competition assays were performed with various known concentrations of dextrorotatory and levorotatory MTM and, in some experiments, CPZ solutions were simultaneously evaluated for their 50% inhibitory concentrations (IC_{50}).

DA receptor studies utilized both [^{3}H]DA (agonist) and [^{3}H]SP (antagonist) binding as described by Creese *et al.* (6, 7). In these experiments, 1 μM (+)-butaclamol was utilized as the cold competitor in samples to define nonspecific binding and to serve as the "blank" in the competition assays. Competition assays contained either 100 nM [^{3}H]DA or 3 nM [^{3}H]SP and varied concentrations of test drugs.

Opiate receptor assays were performed as described by Simantov and Snyder (8). In these experiments we utilized 1 μM levallorphan as the cold competitor to define nonspecific binding. Experiments using [^{3}H]ENK as the opiate ligand were performed in polystyrene tubes to avoid adsorption to glass surfaces, and incubations were carried out either at 0° for 3 hr or (with bacitracin, 50 μM) at 25° for 10 min as employed with the other opiate ligands. Competition assays were performed with the following concentrations of radioactively labeled opioids: [^{3}H]DHM, 1 nM; [^{3}H]ENK, 2 nM; [^{3}H]NAL, 1.5 nM.

Receptor assays for serotonin followed the procedure of Bennett and Snyder (9). Nonspecific binding was defined as that occurring in the presence of 1 μM methysergide. The competition assays incorporated 2 nM [^{3}H]5-HT as the labeled ligand and various concentrations of cold competitors. One alteration of the original technique was incorporation of tissue homogenate preincubation at 37° for 10 min between the two wash steps to allow for enzymatic degradation of endogenous 5-HT (10).

Each assay step was performed in duplicate and each experiment was performed at least twice with separate homogenates. All assays utilized 50 mM Tris buffer systems (pH 7.4 at 37°) with various additional components, depending upon which receptor system was under investigation. All drug solutions were prepared fresh on the day of use in 0.1% ascorbic acid except for opiate receptor assays, in which distilled water was used as the solvent. The opiate receptor assays were performed without ascorbic acid because of the particularly detrimental influence of this compound upon *in vitro* opiate receptors (11). Incubations of appropriate homogenates and the various cold competitors were initiated upon the addition of tritiated ligand, then allowed to attain equilibrium. Reactions were terminated by filtration through Whatman GF/B glass-fiber filters followed by three rapid washes with 5-ml aliquots of ice-cold 50 mM Tris buffer. Filters retaining bound radioactivity were placed in scintillation vials, dried at room temperature overnight, extracted in 10 ml of Aquasol II (New England Nuclear Corporation) at room temperature for 24 hr, then counted in a Beckman LS 8000 liquid scintillation counter at an efficiency of 50%. In the competition assays, specific binding inhibition percentage was calculated from the total binding of the standard radioligand concentration (100% binding) observed without excess unlabeled competitor and with the appropriate blank (0% binding). The percentage of specific binding inhibition was transformed to logit values and plotted against the logarithm of the competitor concentration. These data then allowed calculation of correlation coefficients, the Hill coefficient (n_H) and the IC_{50} value, the concentration required to inhibit specific binding by 50%. Statistical analysis of the competition experiments was performed on the logit binding data by use of ANOVA utilizing $p < 0.05$ as the level of significance.

RESULTS

Scatchard analysis of data from the various saturation assays indicated that these *in vitro* radioligand receptor systems exhibited characteristics similar to those reported by other investigators. Calculated K_D and the B_{max} values were similar to those reported in the original experiments which characterized these receptor types. The regression analysis of all plots indicated a linear,

monophasic binding phenomenon for each radioligand over the concentration range investigated which included that used in subsequent competition assays. Logit transformation of the observed percentage binding data and subsequent regression analysis with the corresponding logarithm of competitor concentration linearized the dose-response curve and facilitated estimation of apparent Hill coefficients and IC_{50} values. These values, calculated from the observed mean binding inhibition data in two or, as noted, three separate experiments, are shown in Table 1. In all cases (−)-MTM was either more effective than, or in opiate receptor systems, equipotent with, (+)-MTM. The quotient of the less active isomer IC_{50} divided by that of the more active isomer yields a parameter estimating stereoselectivity. A stereoselective response is therefore characterized by a ratio significantly greater than 1.0. The ratio of (+)-MTM to (−)-MTM IC_{50} values are also shown in Table 1. It is apparent upon examining the mean data that isomeric differences in receptor interactions are seen with DA and 5-HT receptors, whereas opiate receptors apparently fail to discriminate between (+)- and (−)-MTM. It is interesting to note that, in the DA receptor systems, the (−)-MTM isomer inhibits specific [^{3}H]SP binding more effectively than [^{3}H]DA binding. The (+)- to (−)-MTM activity ratio of 44.18 seen with [^{3}H]SP displacement is much larger than the 8.2 ratio observed with [^{3}H]DA displacement. Also, the stereoselectivity factor of 7.3 seen in [^{3}H] 5-HT binding inhibition by (+)- and (−)-MTM appears to be quite similar to that observed in the [^{3}H]DA experiments.

The apparent Hill coefficients, n_H, shown in Table 1 were calculated as the slopes of the regression lines derived from the competition data. The mean logit binding data for each test drug plotted against the logarithm of its corresponding concentration produce a curve with a slope equivalent to the logarithmic form of the Hill equation (12). The majority of apparent Hill coefficients are less than, or approximately equal to, the integer 1. Notably, (+)-MTM competition with [^{3}H]SP and [^{3}H] NAL exhibit the largest values of n_H, approximately 2-fold greater than that seen with the levorotatory isomer.

Logit binding data from each competition experiment was used in the ANOVA to identify statistically significant drug effects. In the statistical analysis the results of the competition experiments were considered as two groups. The first comparison involved the effects of (+)- and (−)-MTM in each radioligand system examined: two separate groups were analyzed owing to the triplicate DA experiments and duplicates of all others. The coefficients of variance shown in Table 1 indicate that triplicate analyses provided no advantage over duplicate receptor competition experiments. The ANOVA indicated significant isomeric effects over-all in the DA and 5-HT experiments. On subsequent *post hoc* examinations of cell totals in the ligand versus isomer two-way subtables, the dopamine receptors ([^{3}H]DA and [^{3}H]SP) and 5-HT receptors exhibited statistically significant differential isomer effects of stereoselectivity. In the second statistical analysis, the effects of CPZ were compared with (−)-MTM. In both the DA and 5-HT receptor systems, CPZ exhibited properties similar to (−)-MTM. Differences between CPZ and (−)-MTM were found to be nonsignificant at $p < 0.05$ by ANOVA. These results of the statistical analyses are also indicated in Table 1.

In order to address the question of the relevance of the apparent relationship between the IC_{50} of (−)-MTM and the isomeric stereoselectivity ratio, regression analysis

TABLE 1

Summary of radioligand binding inhibition data by methotrimeptrazine enantiomers and chlorpromazine

Mean molar concentrations of test drugs required to inhibit the specific binding of tritiated agonists and antagonists by 50% (IC_{50}) in n different experiments are shown with their respective coefficients of variance (V). Apparent Hill coefficients (n_H) are also presented for each compound. In addition, the stereoselectivity parameter for each receptor type, (+)/(−) IC_{50}, and the ratios of CPZ to (−)-MTM IC_{50} values for the three ligands used in their comparison study are also displayed. Binding data were subjected to ANOVA. The two-way subtables involving each radioligand and paired test drugs were subjected to *F*-tests in order to identify differences between isomer or neuroleptic effects within the appropriate radioligand binding system. Ratios of (+)/(−) or CPZ/(−) IC_{50} values that represent statistically significant ($p < 0.05$) drug differences are designated with an asterisk.

Drug and parameter	Radioligand					
	[^{3}H]DA ($n = 3$)	[^{3}H]SP ($n = 3$)	[^{3}H]DHM ($n = 2$)	[^{3}H]ENK ($n = 2$)	[^{3}H]NAL ($n = 2$)	[^{3}H]5-HT ($n = 2$)
(+)-MTM						
IC_{50}	6.0×10^{-6}	3.0×10^{-6}	1.4×10^{-5}	1.2×10^{-5}	2.7×10^{-5}	5.7×10^{-6}
V	0.98	0.15	<.01	0.45	0.29	0.03
n_H	0.92	1.6	1.0	0.96	1.6	0.79
(−)-MTM						
IC_{50}	7.3×10^{-7}	6.7×10^{-8}	1.3×10^{-5}	9.2×10^{-6}	1.6×10^{-5}	7.8×10^{-7}
V	0.30	0.80	0.58	0.04	0.17	0.02
n_H	0.80	0.77	1.3	1.2	1.2	0.56
(+)/(−)IC_{50}	8.2*	44.8*	1.1	1.3	1.7	7.3*
CPZ						
IC_{50}	1.3×10^{-6}	5.7×10^{-8}	—	—	—	6.1×10^{-7}
V	0.11	0.43				0.21
n_H	0.91	0.72	—	—	—	0.81
CPZ/(−)IC_{50}	1.8	0.9				0.8

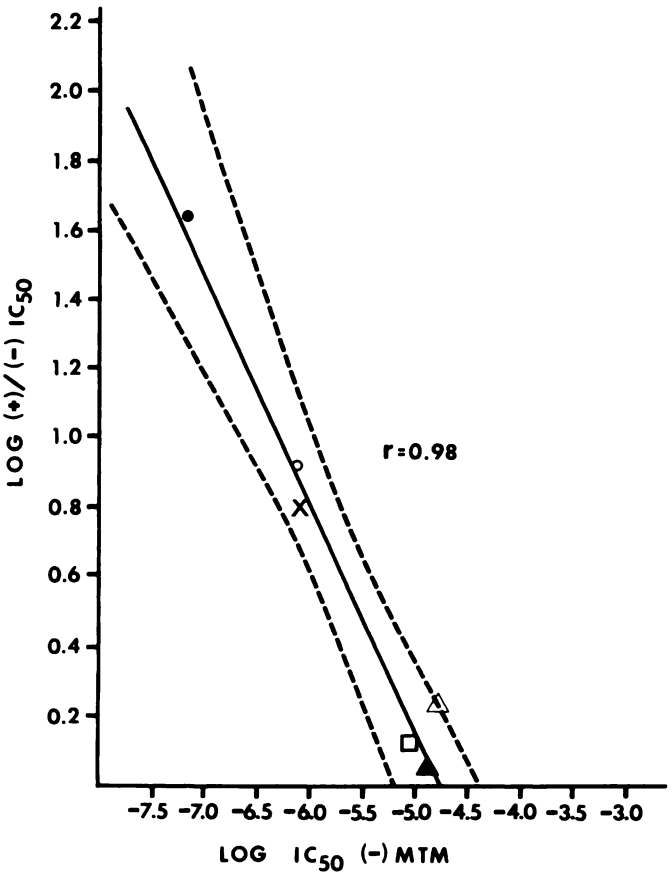

FIG. 1. *Log-log plot of receptor stereoselectivity versus affinity estimators for the interaction of methotrimeprazine enantiomers and six receptor types*

The ordinate value for each point corresponds to the logarithm of the ratio of mean IC_{50} values for (+)-MTM and (−)-MTM. Values on the abscissa are logarithms of the more effective isomer IC_{50}. The *solid regression line* indicates the strong correlation between these parameters, and the *dashed lines* bracketing this curve delineate the 99% confidence interval for this function. Symbols designated at each point correspond to the following radiolabeled receptor ligands: ○, [^{3}H]DA; ●, [^{3}H]SP; ▲, [^{3}H]DHM; □, [^{3}H]5-L-metenkephalin; △, [^{3}H]NAL; ×, [^{3}H]5-HT.

was undertaken. Linear regression of the logarithms of (−)-MTM IC_{50} values and the corresponding (+)- to (−)-MTM activity ratios for each receptor type is shown in Fig. 1. The correlation coefficient of 0.98 is significant at the $p < 0.05$ level.

DISCUSSION

Radioligands used in these experiments were selected to label both different types of receptors as well as putative subtypes of receptors. Tritiated DA and SP were utilized to examine both the agonist and antagonist state of the DA receptor (6, 13, 14). Opiate receptors were studied with an opioid agonist (DHM), an endogenous agonist (5-L-metenkephalin), and an antagonist (NAL) to ensure a thorough examination of potential MTM interactions at multiple receptor subtypes (15). Serotonin receptors were examined only with the tritiated agonist 5-HT. There is some question regarding the ability of this ligand to discriminate between high- and low-affinity receptor populations (16), but it is difficult to identify an ideal serotonin receptor label.

Despite the different tissues and blanking agents used in our opiate and 5-HT receptor assays, quite good agreement was seen between these saturation data and previously reported values. Any small differences in K_D or B_{max} values were attributed to the use of bovine caudate nucleus rather than whole rat brain or cortical tissue. The monophasic Scatchard plots obtained from all saturation studies are compatible with single receptor site labeling by the tritiated ligands in conjunction with the corresponding blanking agent. Specific binding in the various competition experiments was defined within these populations of single receptor sites. It is worthy to note that all the blanking agents were of the most pharmacologically active stereoisomeric configuration, thus defining a stereoselective receptor population. In contrast, some radioligands were possibly racemic mixtures, notably [^{3}H]DA and [^{3}H]5-HT. The relative contributions of the different stereoisomer affinities may also play a role in determining the calculated K_D values (17). It must be borne in mind that, unless all biochemical and stereochemical variables are fully controlled, K_D values are always apparent K_D values. The observed K_D and B_{max} values are sufficiently similar to those previously reported to support the conclusion that the *in vitro* receptor assays were performing adequately to test MTM isomers in competition experiments.

The results of the MTM enantiomer competition studies clearly fall into two categories. Statistically significant stereoselective actions are seen with DA and 5-HT receptors, whereas opiate receptors fail to discriminate between (+)- and (−)-MTM. Dopamine receptors further differentiate between the enantiomers on the basis of whether an agonist or antagonist is being displaced. Although these results may be interpreted as different affinities of the MTM isomers for agonist and antagonist states of the DA receptor (13), they are also compatible with interactions at an accessory binding site as proposed by Ariens *et al.* (18). Dopamine receptors have previously been shown to exhibit a high degree of stereoselectivity for neuroleptic agents (19). Opiate receptors have also been shown to be highly stereoselective (20).

The results from [^{3}H]5-HT binding displacement studies are similar to those of [^{3}H]DA. Both the apparent stereoselectivity and the (−)-MTM IC_{50} values are quite similar at these agonist-labeled sites. This magnitude of antiserotonergic activity is largely in agreement with earlier findings in peripheral tissue bioassay systems (3).

In the three opiate receptor systems studied, it is apparent that the very large IC_{50} values indicate a poor interaction between these receptor species and MTM molecules. The observation that low or even moderate concentrations of (−)-MTM failed to compete successfully with [^{3}H]NAL binding was reported earlier by St. John and Born (4). We have extended and expanded upon their findings to include the poor competition of tritiated agonist binding and the virtually identical effects of (+)-MTM in these *in vitro* systems. On the basis of Pfeiffer's postulate (2) proposing that a high order of stereoselectivity indicates specificity for receptor types, we conclude that (−)-MTM analgesia is not attributable

to direct interaction with opiate receptors owing to the absence of stereoselectivity at these sites for this isomer.

A correlative technique evaluating all of the tritiated ligand displacement data with respect to isomeric differences further lends support to this conclusion. When the active enantiomer IC_{50} [a function of receptor affinity (20)] is plotted against the ratio of inactive to active IC_{50} values (a function of stereoselectivity) on logarithmic axes, a highly significant linear relationship is observed. Using the two MTM enantiomers and these diverse radioligand receptor systems we have identified a strong correlation between receptor affinity estimates and the corresponding stereoselectivity parameters. In short, the degree of stereoselectivity may serve as a predictor of relative pharmacological specificity, at least in these systems.

Interpretation of the calculated apparent Hill coefficients is somewhat difficult. Since no estimate of cooperativity between MTM binding sites is available, it is possible to assume that a high degree of cooperativity exists. In this case the n_H is a conservative estimate of the number of binding sites involved; the next largest integer value corresponding to the true value. The data presented in this report suggest that (+)-MTM may interact with additional binding sites when displacing receptor antagonists.

The final point to be derived from these experiments is that (−)-MTM is similar to CPZ. As shown in Table 1 the active MTM isomer is not significantly different from CPZ in DA or 5-HT receptor systems. In the past it has been assumed that branching of the aliphatic side chain produces a less-active phenothiazine neuroleptic (21). This effect may now be attributed to the generation of a racemic product rather than a general deleterious aliphatic structure preventing optimal drug-receptor interactions.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Leo M. Harvill for advice and assistance with the statistical analyses used. We are also very grateful to Mr. Charles Hawkins, of Valleydale Meat Packers, for his continuing cooperation in supplying tissues for this and other research projects. We also wish to thank Ms. Helen Higdon and Ms. Teresa Garland for typing the manuscript.

REFERENCES

1. Seeman P. Antischizophrenic drugs—membrane receptor sites of action. *Biochem. Pharmacol.* **26**:1741–1748 (1977).
2. Pfieffer, C. C. Optical isomerism and pharmacological action, a generalization. *Science (Wash. D. C.)* **124**:29–31 (1956).
3. Courvoiser, S., R. Ducrot, J. Fournel, and L. Julou. Proprietes pharmacodynamiques generales de la levomepromazine (7.044 R.P.). *C. R. Soc. Biol.* **151**:1378–1382 (1957).
4. St. John, A. B., and C. K. Born. Characterization of analgesic and activity effects of methotrimeprazine and morphine. *Res. Commun. Chem. Pathol. Pharmacol.* **26**:25–34 (1979).
5. Creese, T., D. R. Burt, and S. H. Snyder. Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. *Science (Wash. D. C.)* **192**:481–483 (1976).
6. Creese, I., D. R. Burt, and S. H. Snyder. Dopamine receptor binding: differentiation of agonist and antagonist states with ^{3}H-dopamine and ^{3}H-haloperidol. *Life Sci.* **17**:993–1002 (1975).
7. Creese, I., R. Schneider, and S. H. Snyder. ^{3}H-Spiroperidol labels dopamine receptors in pituitary and brain. *Eur. J. Pharmacol.* **46**:377–381 (1977).
8. Simantov, R., and S. H. Snyder. Morphine-like peptides, leucine enkephalin and methionine enkephalin: interactions with the opiate receptor. *Mol. Pharmacol.* **12**:987–998 (1976).
9. Bennett, J. P., and S. H. Snyder. Serotonin and lysergic acid diethylamide binding in rat brain membranes: relationship to postsynaptic serotonin receptors. *Mol. Pharmacol.* **12**:373–389 (1976).
10. Fuller, R. W., N. R. Mason, and B. B. Malloy. Structural relationships in the inhibition of [^{3}H]serotonin binding to rat brain membranes *in vitro* by 1-phenylpiperazines. *Biochem. Pharmacol.* **29**:833–835 (1980).
11. Leslie, F. M., C. E. Dunlap, and B. M. Cox. Ascorbate decreases ligand binding to neurotransmitter receptors. *J. Neurochem.* **34**:219–221 (1980).
12. Segel, T. *Enzyme Kinetics.* John Wiley & Sons, New York, 353–375 (1975).
13. Riddall, D. R., and W. J. Leavens. Affinities of drugs for the agonist and antagonist states of the dopamine receptor. *Eur. J. Pharmacol.* **51**:187–188 (1978).
14. Lew, J. Y., and M. Goldstein. Dopamine receptor binding for agonists and antagonists in thermal exposed membranes. *Eur. J. Pharmacol.* **55**:429–430 (1979).
15. Chang, K., and P. Cuatrecasas. Multiple opiate receptors. *J. Biol. Chem.* **254**:2610–2618 (1979).
16. Pedigo, N. W., H. T. Yamamura, and D. L. Nelson. Discrimination of multiple [^{3}H]5-hydroxytryptamine binding sites by the neuroleptic spiperone in rat brain. *J. Neurochem.* **36**:220–226 (1981).
17. Burgisser, E., A. A. Hancock, R. J. Lefkowitz, and A. DeLean. Anomalous equilibrium binding properties of high affinity racemic radioligands. *Mol. Pharmacol.* **19**:205–216 (1980).
18. Ariens, E. J., A. J. Beld, J. F. Rodrigues de Miranda, and A. M. Simons. The pharmacon-receptor-effector concept, in *The Receptors: A comprehensive Treatise* (R. D. O'Brien, ed.), Vol. 1. Plenum Press, New York, 33–91 (1979).
19. Seeman, P., K. Westman, M. Protiva, J. Jilek, P. C. Jain, A. K. Saxena, N. Anad, L. Humber, and A. Philipp. Neuroleptic receptors: stereoselectivity for neuroleptic enantiomers. *Eur. J. Pharmacol.* **56**:247–251 (1979).
20. Pert, C. B., and S. H. Snyder. Opiate receptor: demonstration in nervous tissue. *Science (Wash. D. C.)* **179**:1011–1014 (1973).
21. Cheng, Y., and W. H. Prusoff. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes a 50 percent inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **22**:3099–3108 (1973).
22. Ferris, R. M., J. E. Burcsu, and R. A. Maxwell. Stereoisomeric aspects of psychotherapeutic drugs, in *Psychotherapeutic Drugs, Part I; Psychopharmacology Series.* (E. Usdin and I. Forrest, eds.), Vol. 2. Marcel Dekker, New York, 107 (1976).

Send reprint requests to: Dr. T. A. Robert, Department of Pharmacology, Quillen-Dishner College of Medicine, East Tennessee State University, Johnson City, Tenn. 37614.