# Neurotransmitter Receptor Binding Studies Predict Antiemetic Efficacy and Side Effects[1,2]

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**Radioligand binding studies were used to analyze the interaction of antiemetics with central dopamine $D_2$ and alpha-adrenergic$_1$ receptors. The affinity of antiemetics for dopamine $D_2$ receptors labeled by $^3$H-spiperone significantly correlated with clinically effective drug doses ($r = 0.92$; $P < 0.01$). Furthermore, the relative inhibition of alpha-adrenergic$_1$ receptors as measured by $^3$H-WB 4101 binding predicts the clinical side effects of sedation and orthostatic hypotension. Neurotransmitter receptor binding analysis provides a rapid and sensitive technique for measuring antiemetic potency as well as associated side effects. [Cancer Treat Rep 70:637–641, 1986]**

Nausea and vomiting are two of the most untoward side effects of cancer chemotherapy (1–3). To date, a variety of pharmacological agents have been used in the treatment of chemotherapy-induced nausea and vomiting (4–8). In particular, neuroleptics are commonly used therapeutic agents. Theoretically, their efficacy derives from a blockade of dopamine receptors, presumably in the region of the area postrema (9). However, neuroleptics vary in their clinical efficacy and, in addition, are frequently associated with severe side effects such as sedation and orthostatic hypotension.

Radioligand binding studies provide a rapid and sensitive measure of drug potencies at central neurotransmitter receptor binding sites (10,11). For example, the potency of neuroleptics in blocking dopamine $D_2$ receptors correlates significantly with their antipsychotic activity (12). In addition, drug affinity for alpha-adrenergic$_1$ receptors has been used as a predictor of clinical sedation and orthostatic hypotension (13,14). We therefore examined a series of ten antiemetics and three neuroleptics at both dopamine $D_2$ receptors labeled by $^3$H-spiperone and alpha-adrenergic$_1$ receptors labeled by $^3$H-WB 4101. We now report that radioligand binding studies can be used as a rapid biochemical screen of antiemetic efficacy and side effects.

## MATERIALS AND METHODS

Receptor binding assays were performed according to the methods of Peroutka et al (13). Briefly, adult rat brains were purchased from Pel-Freez Biologicals (Rogers, AR) and stored at −20°C. The brains were defrosted and the various anatomical regions were dissected as needed immediately prior to each experiment. Tissues were homogenized in 20 volume of 50 mM Tris-HCl buffer (pH 7.7 at 25°C) using a Brinkmann Polytron and then centrifuged at 49,000 × $g$ for 10 minutes. The supernatant was discarded and the pellet resuspended in the same volume of Tris-HCl buffer prior to a second centrifugation at 49,000 × $g$ for 10 minutes. The final pellet was resuspended in 80 volume of Tris-HCl buffer for $^3$H-WB 4101 binding in the cortex or in 240 volume of Tris-HCl buffer containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$ for $^3$H-spiperone binding in the caudate.

Binding assays consisted of 0.1 ml of $^3$H-ligand (final concentration of 0.5 nM $^3$H-WB 4101 or 0.8 nM $^3$H-spiperone), 0.1 ml of buffer or displacing drug, and 0.8 ml of tissue suspension. This concentration of $^3$H-WB 4101 was used due to the recent observation of Norman et al (15) that higher concentrations of $^3$H-WB 4101 (3 nM) also label 5-hydroxytryptamine$_{1A}$ receptors. Following incubation at 25°C for 30 minutes, the assays were rapidly filtered under vacuum through glass fiber filters (Schleicher and Schuell; #32 glass; Keene, NH) with two 5-ml washes using 50 mM Tris-HCl buffer. Radioactivity was measured by liquid scintillation spectroscopy in 7 ml of Aquasol (New England Nuclear; Boston, MA) at 54% efficiency. Specific binding was defined as the excess over blanks taken in the presence of 1 μM droperidol for $^3$H-WB 4101 binding and 1 μM (+)-butaclamol for $^3$H-spiperone binding. Generally, 75%–80% of the total $^3$H-WB

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4101 binding and 60%–70% of the total $^3$H-spiperone binding was specific.

Drugs were dissolved as follows: amitriptyline, trimethobenzamide, and metoclopramide in 50 mM Tris-HCl buffer; chlorpromazine and trifluoperazine in deionized water; droperidol, haloperidol, thiethylperazine, and fluphenazine were first dissolved in 1–3 ml of 95% ETOH and then diluted in buffer; and (+)-butaclamol, domperidone, pimozide, prochlorperazine, and spiperone were first dissolved in 0.01–1 ml of glacial acetic acid and then diluted in buffer.

Drugs were obtained from the following sources: $^3$H-WB 4101 and $^3$H-spiperone (19.8 Ci/mmol and 23.4 Ci/mmol, respectively; New England Nuclear), amitriptyline, chlorpromazine, and metoclopramide (Sigma Chemical Co, St Louis, MO); droperidol, domperidone, and spiperone (Janssen Pharmaceuticals, Piscataway, NJ); (+)-butaclamol (Research Biochemicals, Inc, Waltham, MA); trifluoperazine and prochlorperazine (Smith, Kline & French, Philadelphia, PA); thiethylperazine (Boehringer Ingelheim Ltd, Ridgefield, CT); and trimethobenzamide (Beecham Laboratories, Bristol, TN).

## RESULTS

The affinities of antiemetic drugs for dopamine $D_2$ receptors in neuronal membranes were determined (table 1). Of the 13 drugs selected for analysis, spiperone and fluphenazine are the most potent agents at the dopamine $D_2$ receptor with affinities of 0.23 and 0.50 nM, respectively. Thiethylperazine, (+)-butaclamol, haloperidol, and droperidol are essentially equipotent with $K_i$ values of 1–2 nM. Trifluoperazine is slightly less potent at this receptor ($K_i$ = 2.3 nM). By contrast, pimozide, prochlorperazine, and domperidone have affinities of approximately 10 nM. Chlorpromazine ($K_i$ = 21 nM) is approximately one order of magnitude less potent than trifluoperazine. Metoclopramide is significantly less potent at the dopamine $D_2$ receptor, with an apparent $K_i$ value of 160 nM. The least potent agent is trimethobenzamide, with a $K_i$ value of 640 nM.

Of the 13 agents analyzed, ten have been previously studied in clinical antiemetic trials. The average recommended clinical dose for each of these ten agents was obtained from the literature (table 1). As shown in figure 1, a significant correlation exists between the average recommended clinical dose and antiemetic potency at the dopamine $D_2$ receptor ($r = 0.92$; $P < 0.01$).

Antiemetic potency was also assessed at central alpha-adrenergic$_1$ receptors labeled by $^3$H-WB 4101. In general, each of these agents also displays high affinity for this neurotransmitter receptor site. As shown in table 2, droperidol ($K_i$ = 1.3 nM) is the most potent agent at the alpha-adrenergic$_1$ receptor. Drugs such as fluphenazine, haloperidol, spiperone, thiethylperazine, and chlorpromazine all display approximately 10 nM affinity for this receptor. By contrast, (+)-butaclamol, trifluoperazine, pimozide, and domperidone are approximately fivefold to tenfold weaker at the $^3$H-WB 4101 binding site. Prochlorperazine is significantly less potent than these agents, with a $K_i$ value of 200 nM. Finally, metoclopramide and trimethobenzamide are essentially inactive at the alpha-adrenergic$_1$ site, with $K_i$ values > 10,000 nM.

A comparison of the relative inhibition of dopamine $D_2$ and alpha-adrenergic$_1$ receptors by chlorpromazine and prochlorperazine is shown in figure 2. As shown in figure 2A, prochlorperazine begins to inhibit the binding of $^3$H-spiperone at the dopamine $D_2$ receptor at concentrations > 3 nM. By concentrations > 10 nM, approximately 50% of the specific $^3$H-spiperone binding has been displaced by prochlorperazine. At 1000 nM, prochlorperazine completely inhibits the binding of $^3$H-spiperone to the dopamine $D_2$ receptor. By contrast, prochlorperazine is less potent in inhibiting the binding of $^3$H-WB 4101 to the alpha-adrenergic$_1$ receptor. No effect on the $^3$H-WB 4101 binding is observed until concentrations > 100 nM are

TABLE 1.—Antiemetic interactions with dopamine $D_2$ receptors

| Drug | Trade name | Recommended clinical dose (mg)* | Potency at dopamine $D_2$ receptor ($K_i$ in nM) |
|---|---|---|---|
| Spiperone | — | — | 0.23 |
| Fluphenazine | Prolixin | 1.5–2 | 0.50 |
| Thiethylperazine | Torecan | 10–20 | 1.1 |
| (+)-Butaclamol | — | — | 1.3 |
| Haloperidol | Haldol | 1–10 | 1.3 |
| Droperidol | Inapsine | 5–10 | 1.9 |
| Trifluoperazine | Stelazine | 1 | 2.3 |
| Prochlorperazine | Compazine | 10–25 | 6.8 |
| Pimozide | Orap | — | 7.8 |
| Domperidone | Motilium | 4–20 | 11 |
| Chlorpromazine | Thorazine | 10–300 | 21 |
| Metoclopramide | Reglan | 70–210 | 160 |
| Trimethobenzamide | Tigan | 200–250 | 640 |

* Clinical data were obtained from ref Nos. 3, 5, 8, and 19.

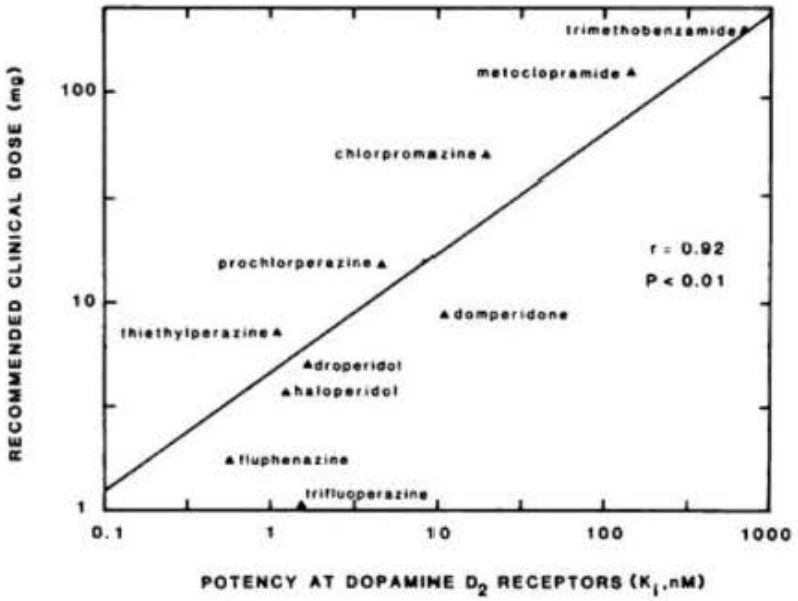



FIGURE 1.—Correlation between antiemetic affinity for dopamine $D_2$ receptors labeled by $^3$H-spiperone and average recommended clinical doses.

achieved. At a concentration of approximately 500 nM, 50% of the specific alpha-adrenergic$_1$ binding is displaced. No residual binding remains in the presence of 10,000 nM prochlorperazine.

An inverse pattern of relative drug potency is observed with chlorpromazine (fig 2B). Chlorpromazine is most potent in inhibiting $^3$H-WB 4101 binding to the alpha-adrenergic$_1$ receptor, with displacement first noted at 1 nM and becoming maximal at 1000 nM. Inhibition (50%) of alpha-adrenergic$_1$ receptor binding occurs at approximately 20 nM. Approximately tenfold higher concentrations of chlorpromazine are needed to achieve the same effect at the dopamine $D_2$ receptor labeled by $^3$H-spiperone.

TABLE 2.—Antiemetic interactions with alpha-adrenergic$_1$ receptors

| Drug | Potency at alpha-adrenergic$_1$ receptors ($K_i$ in nM) | Ratio of $K_i$ alpha-adrenergic$_1$: $K_i$ dopamine $D_2$ * |
|---|---|---|
| Chlorpromazine | 8.8 | 0.42 |
| Droperidol | 1.3 | 0.68 |
| Domperidone | 74 | 6.7 |
| Pimozide | 78 | 10 |
| Haloperidol | 14 | 11 |
| Thiethylperazine | 17 | 15 |
| Fluphenazine | 8.1 | 16 |
| Trimethobenzamide | 16,000 | 25 |
| Prochlorperazine | 200 | 29 |
| Trifluoperazine | 68 | 30 |
| (+)-Butaclamol | 45 | 35 |
| Metoclopramide | 10,000 | 62 |
| Spiperone | 25 | 110 |

* From table 1.

## DISCUSSION

The major finding of the present study is that neurotransmitter receptor binding techniques can be used to screen for both the clinical efficacy and potential side effects of antiemetics. These data extend and confirm the observation of Peroutka and Snyder (11) that antiemetics display high affinity for dopamine $D_2$, histamine, and/or muscarinic cholinergic receptors. Theoretically, the antiemetic effects of neuroleptics derive from their blockade of dopamine receptors in the chemoreceptor trigger zone (16,17). In particular, dopamine $D_2$ receptors have been identified in the area postrema (9). These receptors can be directly labeled using $^3$H-spiperone as a radioligand in neuronal membrane preparations. Since the affinities of antiemetics for these receptors significantly correlate with their effective clinical doses ($r = 0.92$; $P < 0.01$), these data confirm that the dopamine $D_2$ receptor mediates antiemetic actions.

The ability to predict antiemetic efficacy based on radioligand data has many practical implications. First of all, drugs such as spiperone, pimozide, and (+)-butaclamol are currently unavailable for clinical use in the United States. However, the data in the present study

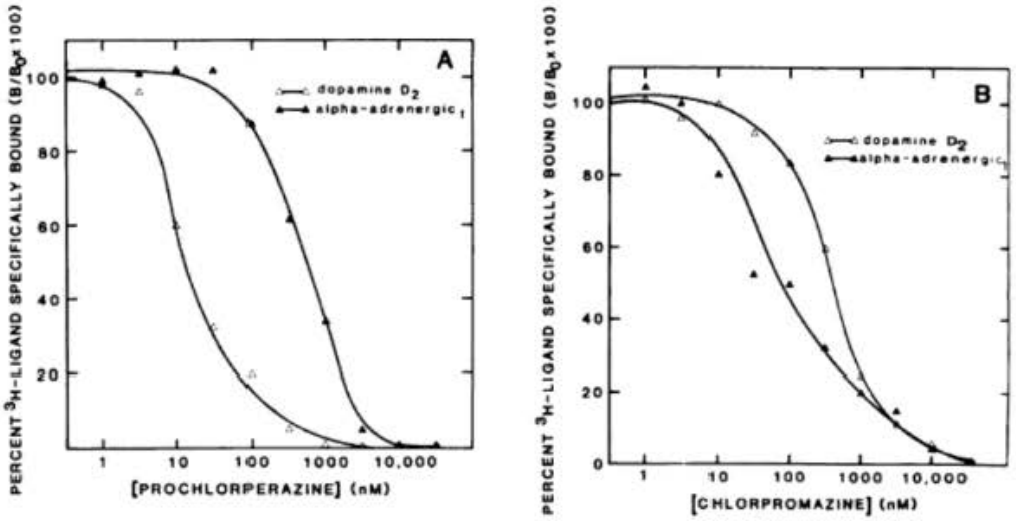



FIGURE 2.—Inhibition of dopamine $D_2$ receptors labeled by $^3$H-spiperone and alpha-adrenergic$_1$ receptors labeled by $^3$H-WB 4101. A (inhibition by prochlorperazine) and B (inhibition by chlorpromazine).

suggest that these agents should be potent antiemetics. Second, the potency of novel antiemetics at the dopamine $D_2$ receptor may be a useful predictor of effective doses in pilot clinical trials. Third, radioligand studies may be useful in determining the primary site of drug action. For example, the therapeutic efficacy of metoclopramide has been attributed to its effects on gastric motility (18), while the mechanism of action of trimethobenzamide is unclear (2-8). The current data suggest that dopamine receptor blockade plays a prominent, if not primary, role in the antiemetic effects of these two drugs.

At the same time, the interactions of drugs with alpha-adrenergic$_1$ receptors have been correlated with sedative and orthostatic hypotensive side effects (13,14). These side effects have also been noted with antiemetics. Clinical studies have shown that the incidence of orthostatic hypotension and sedation varies widely among antiemetics. For example, chlorpromazine and prochlorperazine are essentially equipotent antiemetics. However, the incidence of sedative and orthostatic side effects with chlorpromazine is far more frequent and severe than that observed with prochlorperazine (8,19).

The use of $^3$H-WB 4101 to label the alpha-adrenergic$_1$ receptors appears to be a useful tool for the analysis of these effects. The relative potencies of drugs at alpha-adrenergic$_1$ and dopamine $D_2$ receptors may explain the variability of the side effects. For example, chlorpromazine is more than twice as potent at alpha-adrenergic$_1$ receptors than at dopamine $D_2$ receptors. At clinically effective antiemetic doses, dopamine $D_2$ receptors are theoretically blocked by chlorpromazine. At such concentrations, an even greater percentage of alpha-adrenergic$_1$ receptors are simultaneously blocked. By contrast, at concentrations of prochlorperazine at which a significant proportion of dopamine $D_2$ receptors are blocked (10 nM), only a small percentage of alpha-adrenergic$_1$ sites are blocked. Thus, the ratio of alpha-adrenergic$_1$ to dopamine $D_2$ affinities can be used as a measure of sedation and orthostatic hypotension. Based on these data, chlorpromazine and droperidol should be relatively sedative, whereas trifluoperazine and metoclopramide should be less sedating. These predictions have been confirmed by clinical experience (8,19-21).

Therefore, radioligand binding studies may provide a useful and accurate laboratory predictor of clinical antiemetic efficacy. In particular, binding studies could be used as a screening device for novel antiemetics that act through dopamine $D_2$ receptors. These techniques may also be applied to the study of antiemetics which act at histamine and muscarinic cholinergic receptors. The experiments could be performed rapidly and economically. Moreover, receptor binding studies can also be used to predict major side effects. Finally, the use of receptor binding assays in the analysis of antiemetic action may provide important information concerning the pathophysiology of nausea and vomiting in humans.

## REFERENCES

1. MORRAN C, SMITH DC, ANDERSON DA, ET AL. Incidence of nausea and vomiting with cytotoxic chemotherapy: a prospective randomized trial of antiemetics. Br Med J 1:1323-1324, 1979.

2. LASZLO J. Nausea and vomiting as major complications of cancer chemotherapy. Drugs 25 (suppl 1):1-7, 1983.
3. FIOTAK S, and MOERTEL CG. Management of nausea and vomiting in the cancer patient. JAMA 245:393-396, 1981.
4. SEIGEL LJ, and LONGO DL. The control of chemotherapy-induced emesis. Ann Intern Med 95:352-359, 1981.
5. WOOD CD. Antimotion sickness and antiemetic drugs. Drugs 17:471-479, 1979.
6. LASZLO J. Treatment of nausea and vomiting caused by cancer chemotherapy. Cancer Treat Rev 9 (suppl B):3-9, 1982.
7. PENTA J, PORTER D, and BRUNO S. The pharmacologic treatment of nausea and vomiting caused by cancer chemotherapy: a review. *In* Antiemetics and Cancer Chemotherapy (Laszlo J, ed). Baltimore, Williams and Wilkins, 1983, pp 53-92.
8. NEIDHART JA, and COGAN M. Experimental antiemetic agents (other than the cannabinoids and metoclopramide). *In* Antiemetics and Cancer Chemotherapy (Laszlo J, ed). Baltimore, Williams and Wilkins, 1983, pp 142-163.
9. STEFANINI E, and CLEMENT-CORMIER Y. Detection of dopamine receptors in the area postrema. Eur J Pharmacol 74:257-260, 1981.
10. SNYDER SH. Drugs and neurotransmitter receptors in the brain. Science 224:22-31, 1983.
11. PEROUTKA SJ, and SNYDER SH. Antiemetics: neurotransmitter binding predicts therapeutic actions. Lancet 2:658-659, 1982.
12. CREESE I, BURT DR, and SNYDER SH. Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. Science 192:481-483, 1976.
13. PEROUTKA SJ, U'PRICHARD DC, GREENBERG DA, ET AL. Neuroleptic drug interactions with norepinephrine alpha receptor binding sites in rat brain. Neuropharmacology 16:549-556, 1977.
14. U'PRICHARD DC, GREENBERG DA, SHEEHAN PP, ET AL. Tricyclic antidepressants: therapeutic properties and affinity for alpha-noradrenergic receptor binding sites in the brain. Science 199:197-199, 1978.
15. NORMAN AB, BATTAGLIA G, MORROW AL, ET AL. $^3$H-WB-4101 labels $S_1$ serotonin receptors in rat cerebral cortex. Eur J Pharmacol 106:461-462, 1985.
16. BORISON HL, and WANG SC. Physiology and pharmacology of vomiting. Pharmacol Rev 5:193-230, 1953.
17. BORISON HL, and MCCARTHY LE. Neuropharmacology of chemotherapy-induced emesis. Drugs 25 (suppl 1):8-17, 1983.
18. SCHULZE-DELRIEU K. Metoclopramide. N Engl J Med 305:28-33, 1981.
19. BYCK R. Drugs and the treatment of psychiatric disorders. *In* The Pharmacological Basis of Therapeutics (Goodman LS, and Gilman A, eds). New York, Macmillan, 1975, pp 170-171.
20. LUCAS VS. Phenothiazines as antiemetics. *In* Antiemetics and Cancer Chemotherapy (Laszlo J, ed). Baltimore, Williams and Wilkins, 1983, pp 93-107.
21. GRALLA RJ, TYSON LB, BORDIN LA, ET AL. Antiemetic therapy: a review of recent studies and a report of a random assignment trial comparing metoclopromide with delta-9-tetrahydrocannabinol. Cancer Treat Rep 68:163-172, 1984.