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Differential interactions of traditional and novel antiemetics with dopamine D_2 and 5-hydroxytryptamine$_3$ receptors

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Summary. The affinities of 11 drugs for both dopamine D_2 and 5-hydroxytryptamine$_3$ (5-HT_3) receptor sites were determined in brain membranes. The five "traditional" antiemetics (chlorpromazine, prochlorperazine, droperidol, fluphenazine, and domperidone) displayed high affinity (<20 n*M*) for dopamine D_2 receptors in corpus striatum but were inactive at 5-HT_3 receptors. In contrast, five recently developed 5-HT_3 antagonists (BRL 43694, ICS 205-930, zacopride, Lilly 278584, and MDL 72222) displayed nanomolar affinity for the 5-HT_3 site but were inactive (>10,000 n*M*) at the dopamine D_2 receptor. Metoclopramide was unique among these agents in that it was similarly potent at dopamine D_2 (240±60 n*M*) and 5-HT_3 (120±30 n*M*) receptors.

Introduction

Nausea and vomiting resulting from cancer chemotherapy are common side effects that can cause patients to refuse subsequent chemotherapeutic sessions [19]. However, against certain types of chemotherapy- or radiation-induced nausea and vomiting, traditional antiemetics such as dopamine D_2 receptor antagonists [27, 30, 33] are only minimally effective. Moreover, dopamine D_2 antagonists often cause side effects such as extrapyramidal symptoms, which further restrict their usefulness [3, 17, 21].

A new class of pharmacological agents has recently been developed that appears to possess uniquely potent and effective antiemetic activity [24]. These drugs have been designated 5-hydroxytryptamine$_3$ (5-HT_3) receptor antagonists and include drugs such as ICS 205-930 and MDL 72222 [12, 28]. Metoclopramide, traditionally considered to be a dopaminergic antagonist, has also been reported to have 5-HT_3 receptor antagonist properties [13, 22]. In animals, 5-HT_3 antagonists block both cytotoxic drug- and radiation-induced emesis [1, 6, 11, 22–24, 31]. These drugs also appear to abolish nausea and vomiting in patients receiving cytotoxic drugs [4, 10, 20]. In the present study, a series of five traditional dopamine D_2 antagonists, five recently developed 5-HT_3 antagonists, and metoclopramide were analyzed at both dopamine D_2 and 5-HT_3 receptor binding sites in brain membranes.

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Material and methods

Radioligand-binding studies were carried out according to the methods of Ison and Peroutka [18] and Peroutka and Hamik [26]. Frozen rat brains (Pel Freez Biologicals; Rogers, Ark) were thawed from −20° C and dissected. Tissue was homogenized in 20 vol. 50 m*M* TRIS (pH 7.7 at 25° C) for [^{3}H]-spiperone binding or Krebs-HEPES buffer for [^{3}H]-quipazine assays and centrifuged at 49,000 *g* for 10 min. Cortical tissue was used for [^{3}H]-quipazine binding and corpus striatum, for [^{3}H]-spiperone binding. The supernatant was discarded and the pellet resuspended in the same volume of buffer. After a 10-min incubation at 37° C, the tissue was again centrifuged. The final pellet was resuspended in 80 vol. assay buffer. For dopamine binding ([^{3}H]-spiperone +40 n*M* ketanserin [25]), the assay buffer consisted of 10^{-5} *M* pargyline, 4 m*M* $CaCl_2$, and 0.1% ascorbate in 50 m*M* TRIS. [^{3}H]-Quipazine binding was carried out in a Krebs-HEPES buffer that consisted of 25 m*M* HEPES, 120 m*M* NaCl, 2.5 m*M* $CaCl_2$, 4.8 m*M* KCl, and 1.2 m*M* $MgCl_2$ (pH adjusted to 7.4).

Binding assays consisted of 0.1 ml [^{3}H]-ligand, 0.1 ml buffer or displacing drug, and 0.8 ml tissue homogenate. Following a 30-min incubation at 25° C, the assays were rapidly filtered under vacuum through #32 glass-fiber filters (Schleicher and Schuell; Keene, NH) with two 5-ml washes using 50 m*M* TRIS buffer. Radioactivity was measured by liquid scintillation spectroscopy in 2.5 ml scintillation cocktail (Research Products International; Mt. Prospect, Ill) at 61% efficiency. All experiments were carried out in triplicate and repeated 3–6 times. Specific binding was defined as the excess over blanks taken in the presence of 10^{-6} *M* (+)butaclamol for [^{3}H]-spiperone binding and 10^{-7} *M* ICS 205-930 for [^{3}H]-quipazine binding. Generally, 75% of the [^{3}H]-spiperone binding and 40% of the [^{3}H]-quipazine binding was specific.

Drugs were prepared for experiments as follows: endo-*N*-(9-methyl-9-azabicyclo-[3,2,1]non-3-yl)-1-methyl-1H-indazole-3-carboxamide (BRL 43694), (3a-tropanyl)-1H-indole-3-carboxylic acid ester (ICS 205-930), zacopride, 1-methyl-*N*-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-1H-indazole-3-carboxamide (Lilly 278584), and metoclopramide were dissolved in assay buffer; 1aH,3a,5aH-tropan-3-yl-3,5-dichlorobenzoate (MDL 72222), chlorpromazine, and ketanserin were dissolved in dH_2O and then diluted in assay buffer; (+)butaclamol, droperidol, and fluphenazine were dissolved in 25%–50% EtOH at

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10^{-3} *M* and then diluted in buffer; and domperidone and prochlorperazine were dissolved in 25% 1 *M* acetic acid and diluted in assay buffer.

Drugs and chemicals were obtained from the following sources: [^{3}H]-quipazine (52.3 Ci/mmol) and [^{3}H]-spiperone (21.4 Ci/mmol), Dupont New England Nuclear (Boston, Mass); BRL 43694, Beecham (Betchworth, England); ICS 205-930, Sandoz Pharmaceuticals (East Hanover, NJ); zacopride, A. H. Robins (Richmond, Va); Lilly 278584, Lilly Research Laboratories (Indianapolis, Ind); metoclopramide, chlorpromazine, TRIS-HCl, HEPES, and pargyline, Sigma Chemical Co. (St. Louis, Mo); prochlorperazine, Smith Kline and French (Philadelphia, Pa); droperidol, domperidone, and ketanserin, Janssen Pharmaceutica (New Brunswick, NJ); (+)butaclamol, Research Biochemicals Inc. (Wayland, Mass); $CaCl_2$, Fisher Scientific (Phillipsburg, NJ); ascorbic acid and NaCl, Mallinckrodt (Paris, Ky); and $MgCl_2$ and KCl, J. T. Baker Chemical Co. (Phillipsburg, NJ).

Results

Drug interaction with D_2 receptors

The results of drug competition studies are given in Table 1. The traditional antiemetics were, as previously reported [18], quite potent at the dopamine D_2 site; K_i values ranged from 2.4 ± 0.6 n*M* for droperidol to 18 ± 5 n*M* for chlorpromazine. Metoclopramide demonstrated a K_i value of 240 ± 60 n*M* ($n = 10$). By contrast, putative 5-HT_3 antagonists were inactive at the dopamine D_2 site in the rat corpus striatum. BRL 43694, ICS 205-930, zacopride, Lilly 278584, and MDL 72222 all demonstrated K_i values of $> 10{,}000$ n*M*. Representative drug competition curves for ICS 205-930, metoclopramide, and domperidone against specific dopamine D_2 receptors are shown in Fig. 1 A.

Drug interactions with 5-HT_3 receptors

Drug affinities were determined at 5-HT_3 membrane-recognition sites labeled by [^{3}H]-quipazine. Of the 11 drugs analyzed, 4 demonstrated less than nanomolar affinity for the 5-HT_3 site. These drugs (BRL 43694, ICS 205-930, zacopride, and Lilly 278584) were essentially equipotent at the 5-HT_3 site, with K_i values ranging from 0.30 to 0.52 n*M*. MDL 72222 was slightly less potent, with a K_i value of 9.2 ± 1 n*M*. Metoclopramide was the only traditional antiemetic that displayed less than micromolar affinity for the 5-HT_3 site, with a K_i value of 120 ± 30 n*M*. In contrast, the remainder of the traditional antiemetics were significantly less potent, with K_i values ranging from $1{,}800 \pm 300$ n*M* for prochlorperazine to $> 10{,}000$ n*M* for fluphenazine and domperidone. Representative drug competition curves are shown in Fig. 1 B.

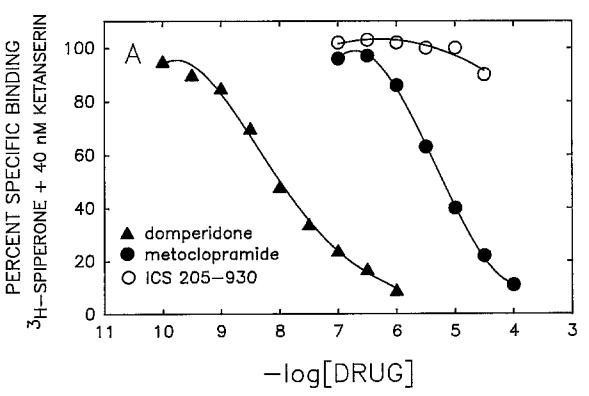

Fig. 1 A. Drug competition studies vs specific [^{3}H]-spiperone + 40 *M* ketanserin binding in rat corpus striatum. Radioligand-binding assays were carried out as described in *Materials and methods.* Nonspecific binding was determined in the presence of 1 μ*M* (+) butaclamol. Data shown are the results of a single experiment carried out in triplicate. Each experiment was repeated 3–6 times

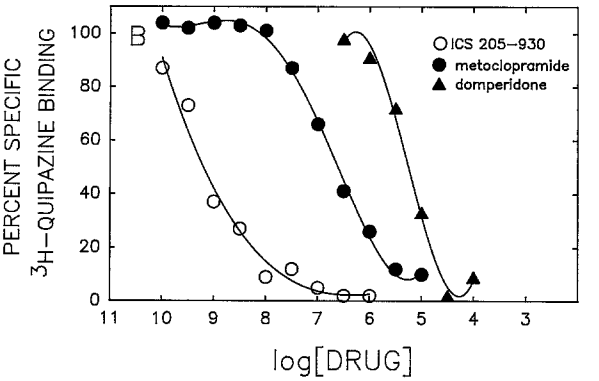

Fig. 1 B. Drug competition studies vs specific [^{3}H]-quipazine binding in rat cortical membranes. Radioligand-binding assays were carried out as described in *Materials and methods.* Nonspecific binding was determined in the presence of 100 n*M* ICS 205-930. Data shown are the results of a single experiment carried out in triplicate. Each experiment was repeated 3–6 times

Table 1. Drug affinities for 5-HT_3- and dopamine D_2-labeled sites in rat brain membranes

Drug	Potency at 5-HT_3 Receptors K_i (n*M*)	Potency at D_2 Receptors K_i (n*M*)	Ratio 5-HT_3/D_2
Dopamine drugs:			
Prochlorperazine	$1{,}800 \pm 300$	7.3 ± 1	200
Chlorpromazine	$1{,}900 \pm 200$	18 ± 5	100
Droperidol	$4{,}200 \pm 30$	2.4 ± 0.6	2,000
Fluphenazine	$> 10{,}000$	4.8 ± 3	$> 2{,}000$
Domperidone	$> 10{,}000$	12 ± 3	> 800
5-HT_3 drugs:			
BRL 43694	0.30 ± 0.04	$> 10{,}000$	< 0.001
ICS 205-930	0.38 ± 0.02	$> 10{,}000$	< 0.001
Zacopride	0.42 ± 0.2	$> 10{,}000$	< 0.001
Lilly 278584	0.52 ± 0.2	$> 10{,}000$	< 0.001
MDL 72222	9.20 ± 1.0	$> 10{,}000$	< 0.002
Mixed drug:			
Metoclopramide	120 ± 30	240 ± 60	0.5

Radioligand-binding studies were carried out as described in *Materials and methods.* Values represent the mean ± SE of 3–6 experiments carried out in triplicate

Discussion

The major finding of the present study is that the 11 drugs tested showed differential interactions with dopamine D_2 and 5-HT_3 receptor sites. The 5-HT_3 antagonists, although showing nanomolar affinity for central 5-HT_3 recognition sites, were inactive at dopamine D_2 receptor sites. Conver-

sely, the traditional antiemetics showed high affinity for the dopamine D_2 site. With the exception of metoclopramide, these drugs were approximately 100–2,000 times more potent at the D_2 site than at the 5-HT_3 site. Metoclopramide appeared to be similarly potent at D_2 and 5-HT_3 sites, a unique finding among the 11 drugs tested.

The antiemetic efficacy of various drugs, including dopamine D_2 antagonists, antihistamines, anticholinergics, and corticosteroids has been well documented [14, 15, 30, 32–34]. Unfortunately, these traditional antiemetics provide only modest relief from nausea and vomiting in patients undergoing therapy with most chemotherapeutic drugs; they are of extremely limited value with agents such as cisplatin, doxorubicin, and dacarbazine, which induce severe nausea and vomiting. Of particular interest is the claim that high-dose metoclopramide is the most effective agent currently available for the treatment of adverse effects caused by these regimens [33].

In contrast, 5-HT_3 antagonists (MDL 72222, ICS 205-930, BRL 24924, and GR38032F) are a recently developed group of drugs that have been shown to be potent antiemetics in ferrets receiving cisplatin and total-body radiation [6, 7, 8, 22–24, 31]. In recent human trials, the 5-HT_3 antagonists GR38032F [10], ICS 205-930 [20], and BRL 43694 [4] provided excellent relief from chemotherapy-induced nausea and vomiting. For example, 14 of 15 patients did not experience nausea or vomiting when given GR38032F along with cytotoxic drugs [10]. The only adverse effects were headache and mild sedation with ICS 205-930 and dry mouth and mild sedation with GR38032F. Therefore, 5-HT_3 antagonists appear to be extremely effective antiemetics when given with strongly emetic anticancer agents.

The mechanism(s) by which the drugs examined in this study relieve nausea and vomiting are not completely known [2]. Specific dopamine D_2 antagonists are thought to act via central dopamine antagonism in the chemoreceptor trigger zone [15]. Metoclopramide is thought to act at the cortex as well as with receptors in the periphery, where it induces gastric motility and emptying [16, 29]. This peripheral action may be explained in part by antagonism of dopamine D_2 receptors. However, since the role of dopamine in the control of gut motility seems minor, other mechanisms must exist through which metoclopramide exerts these effects [29]. It seems likely that this drug interacts with 5-HT_3 receptors located in the enteric system, and it may mediate gastric effects through this mechanism. In addition, since metoclopramide has relatively high affinity for 5-HT_3 recognition sites in rat brain tissues, some of its antiemetic effects may be due to its interactions with these CNS sites.

The antiemetic effects of the 5-HT_3 antagonists appear to be a consequence of 5-HT_3 blockade; however, whether this is due to largely peripheral actions or to both peripheral and central interaction with the receptor remains unclarified. For example, at small doses BRL 24924 (a potent stimulant of gastric motility [5]) mimics abdominal vagotomy in total-body-irradiated ferrets; that is, retching and vomiting is delayed by 30 min. Higher doses of BRL 24924 almost eliminate retching and vomiting for the entire 90-min test period [1]. These results were interpreted as indicating that BRL 24924 may, in addition to its probable action at the abdominal vagi, have an important effect in another area of the body.

In addition to their direct effect on 5-HT transmission, the 5-HT_3 antagonists may also have some modulatory effects on the dopaminergic system. A potent and highly selective 5-HT_3 antagonist, GR38032F, has been shown to modulate hyperactivity resulting from dopamine administration to rats and marmosets [9]. GR38032F was hypothesized to work by interrupting a 5-HT-dopamine-5-HT loop by which 5-HT serves to facilitate dopaminergic transmission. Perhaps a similar interaction between 5-HT and dopamine transmission is involved in the reduction of nausea and vomiting by 5-HT_3 antagonists.

However, the *direct* antagonism of dopamine D_2 receptors does not appear to be necessary for effective antiemetic treatment. Metoclopramide, which is significantly less potent at dopamine D_2 receptors than are the other traditional dopamine D_2 antiemetic agents, has also been shown to be the most effective antiemetic of the group [33]. Recent trials suggest that the 5-HT_3 antagonists are at least as effective as metoclopramide in reducing or eliminating nausea and vomiting resulting from chemotherapy. Thus, it is not apparent that the dopamine D_2 antiemetic effects of metoclopramide are a necessary component of its antiemetic efficacy; its 5-HT_3 antagonism alone may be sufficient to control emesis effectively.

Finally, radioligand-binding studies may be used as a screening tool for the selection of clinically useful drugs. For example, in the present study, two of the 5-HT_3 antagonists (zacopride and Lilly 278584) were not shown to have antiemetic effects. These drugs showed a binding pattern similar to those of the demonstrated antiemetics ICS 205-930, MDL 72222, and BRL 43694; that is, high affinity for the 5-HT_3 site in cortical tissue and inactivity at the dopamine D_2 site in corpus striatum. Zacopride shows an affinity for the 5-HT_3 site similar to that of BRL 43694. Lilly 278584 is more potent at the site than is MDL 72222. Thus, we would predict that both of these drugs would be effective in reducing emesis caused by agents such as cisplatin or by total-body radiation.

In summary, this study demonstrates that affinities of drugs to 5-HT_3 and dopamine D_2 sites correlates with their efficacy as antiemetics. Metoclopramide is superior to the other traditional agents in its antiemetic action and is also the only traditional agent that displays moderate affinity for 5-HT_3 receptors. Clinical trials with the 5-HT_3 antagonists MDL 72222, ICS 205-930, BRL 43694, and GR38032F have shown them to be at least equal to, and probably superior to, metoclopramide in antiemetic efficacy. These data indicate that the 5-HT_3 receptor plays a novel and important role in the pathophysiology of nausea and vomiting.

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