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Interaction of the antiemetic metopimazine and anticancer agents with brain dopamine D_2, 5-hydroxytryptamine$_3$, histamine H_1, muscarine cholinergic and α_1-adrenergic receptors

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Abstract. The interactions of the antiemetic metopimazine (MPZ) and of the chemotherapeutic agents, cisplatin, carboplatin, doxorubicin, etoposide and vincristine were investigated at five neurotransmitter receptor binding sites. MPZ had nanomolar affinity for α_1, dopamine D_2 and histamine H_1 receptors, weak affinity for muscarinic cholinergic receptors, but no affinity for 5-hydroxytryptamine$_3$ (5-HT$_3$) receptors. Except for vincristine, which showed nanomolar affinity for muscarinic cholinergic receptors, none of the chemotherapeutic agents showed affinity for any of the receptors investigated at concentrations ranging between 10^{-5} and 10^{-7} *M*. Accordingly, chemotherapy-induced nausea and vomiting seems to be mediated by mechanisms other than the direct interaction of cytostatics with the neurotransmitter receptors investigated. Our finding that MPZ is without affinity for 5-HT$_3$ receptors and therefore seems to mediate its antiemetic effect predominantly by dopamine D_2 receptor blockade makes it an interesting drug for use in combinations with the new class of antiemetics, the 5-HT$_3$ receptor antagonists. Data obtained in a recent clinical trial support this observation.

Introduction

The physiology of chemotherapy-induced nausea and vomiting is generally unknown. The concept of a coordinating region in the medulla oblongata, the vomiting center (VC), has persisted for 100 years [44], although the existence of a well-defined anatomical region coordinating emesis has recently been questioned [4, 28]. The VC may be activated by abdominal visceral afferents, the chemoreceptor trigger zone (CTZ), the vestibular apparatus and higher brain stem and cortical areas [37]. Positioned in the area postrema (AP) at the caudal end of the fourth ventricle, the CTZ is located outside the blood-brain barrier and is therefore highly specialized to sense blood- and cerebrospinal fluid-borne emetic agents [24]. The significance of the CTZ as a mediator in nausea and vomiting was first described by Borison and Wang [3] and afterwards supported by CTZ-ablation trials [26, 27] and by demonstration of emetic activity for intra-cerebroventricular (ICV) cisplatin [40]. Dopamine D_2, histamine H_1, muscarinic cholinergic and opiate binding sites have been demonstrated in the AP [24], and histamine H_1 [33] and muscarinic cholinergic [47] binding sites have also been detected in areas in close relation to the VC. 5-Hydroxytryptamine$_3$ (5-HT$_3$) receptor binding sites have been demonstrated on neurones in the periphery [11] and in the central nervous system [23]. Although 5-HT$_3$ receptors were found in the AP, the highest concentration was seen in the nucleus tractus solitarius (NTS) [35]. This finding has brought into question the old CTZ-ablation and ICV trials [17] and has brought up the importance of the NTS in the pathophysiology of emesis [35].

5-HT$_3$ receptors seem to be located on abdominal vagal afferents terminating in the AP and in the NTS [25], and it has been suggested that cytotoxic drugs evoke emesis by somehow activating peripheral visceral (vagal) and central vagal afferent fibres in the NTS [2]. It has not yet been clarified how chemotherapeutic agents induce emesis. The most simple explanation would be a direct action of the agents on neuronal systems, as occurs with the emetics apomorphine and dopamine [5]. However, this presupposes that cytotoxic agents act directly at receptors or cause release of neurotransmitters. Although the previously mentioned CTZ-ablation trials and the emetic effect of ICV cytostatics have led to the assumption that chemotherapeutic agents may act directly on the AP, this has never been confirmed by demonstration of a change in neuronal activity in this region [1].

Metopimazine (MPZ) is a derivative of phenothiazine and, as such, is a dopamine D_2 antagonist. High doses seem to improve the antiemetic efficacy [7, 46]. Whether this

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Table 1. Summary of the in vitro binding/filtration techniques used

Receptor	Ligand	Concentration (n*M*)	Tissue	Mg tissue/ sample	Buffer	pH	Incubation temperature (°C)	Incubation time (min)	Filters	Number of washes	Nonspecific binding in the presence of	References
D_2	[^{3}H]-Spiperone	0.5	Corpus striatum	3.08	50 m*M* K-phosphate	7.4	37	15	GF/F	3 2	6,7-ADTN, 10^{-5} *M*	[18] [19]
α_1	[^{3}H]-Prazosin	0.25	Whole brain	10	50 m*M* TRIS	7.7	25	20	GF/F	2	Prazosin, 10^{-6} *M*	[20] [38]
5-HT_3	[^{3}H]-Ly 278 584	1.0	Cortex	16	50 m*M* TRIS	7.7	25	30	GF/C	2	Metoclopramide, 10^{-4} *M*	[49]
H_1	[^{3}H]-Mepyramine	2.0	Whole brain	20	50 m*M* K-phosphate	7.4	25	20	GF/F	3	Promethazine, 2×10^{-6} *M*	[12]
Muscarine	[^{3}H]-QNB	0.12	Whole brain crude homogenate	0.05	10 m*M* Na, K-phosphate	7.4	37	30	GF/B	3	Atropine, 2×10^{-5} *M*	[48]

[^{3}H]-QNB, 1-Quinuclidinyl-[phenyl-4-^{3}H]-benzilate

effect is due to the drug's affinity for the dopamine D_2 receptor, as seen with high-dose prochlorperazine [6], or to its 5-HT_3 receptor affinity, as seen with high-dose metoclopramide [15, 32], is unknown. The efficacy and the observation that MPZ has a very low incidence of extrapyramidal adverse reactions, even when given at high doses [46], makes it an interesting antiemetic.

The first aim of this study was therefore to examine the possible binding of the chemotherapeutic agents cisplatin, carboplatin, doxorubicin, etoposide and vincristine to dopamine D_2, 5-HT_3, muscarinic cholinergic, histamine H_1 and α_1-adrenergic receptors. All of these agents are active in their parent form and were chosen with regard to their emetic potential, that of cisplatin and carboplatin being high; that of doxorubicin, moderate; and that of etoposide and especially vincristine, low [8]. Secondly we investigated the possible binding affinity of MPZ for the same five receptors.

Materials and methods

Male Wistar rats [Mol: Wist, specific pathogen-free (SPF); 170–290 g] were used. Inhibition of the binding of tritium-labeled ligands to different receptors in rat brain membranes was measured using standard in vitro binding/filtration techniques [12, 18–20, 38, 48, 49] (Table 1).

Drug concentrations required to inhibit receptor binding by 50% (IC_{50} values) were estimated from concentration-effect curves using a log-concentration scale. Two experiments were performed with each drug. If concentration-effect curves could be measured, five concentrations of test drug in triplicate, covering three decades, were used. In a series of 100 determinations (historical data) the variance of the log ratio (log R) between the double determinations [Σ (log R)^2/2n] was determined. In case the log R for two determinations for the test drug was greater than that corresponding to 3 SD (99% confidence interval) or 2 SD (95% confidence interval) for muscarinic binding, extra determinations were performed and outliers, discarded. The following antilog (SD) values were obtained: D_2, 1.5; α_1, 1.5; 5-HT_3, 1.4; H_1, 1.5; muscarine, 1.5.

The radioactive ligands [phenyl-4-^{3}H]-spiperone (sp. act., 29 and 99 Ci/mmol), [pyridinyl-5-^{3}H]-pyrilamine (sp. act., 25–28 Ci/mmol) and 1-Quinuclidinyl-[phenyl-4-^{3}H]-benzilate (sp. act., 41.5 Ci/mmol) were supplied by Amersham. [Furoyl-5-^{3}H]-prazosin (sp. act., 18 and 76 Ci/mmol) was obtained from Dupont. [^{3}H]-Ly 278584 (sp. act., 84 Ci/mmol) was kindly donated by Lilly (Dr. D. W. Robertson) via Amersham.

The cytostatics used were cisplatin (Platin), carboplatin (Paraplatin), etoposide (Vepesid; all obtained from Bristol-Myers Squibb), doxorubicin (Adriamycin, supplied by Farmitalia Carlo Erba) and vincristine (Oncovin, obtained from Lilly). MPZ (Vogalene) was obtained from Rhone-Poulenc Rorer A/S. All experimental compounds were dissolved/diluted in distilled water at the day of assay. Each chemotherapeutic agent was investigated for receptor binding affinity at concentrations ranging between 10^{-5} and 10^{-7} *M*. MPZ was investigated at concentrations ranging between 10^{-4} and 10^{-10} *M*. These concentrations correlated well with or, in most cases, even exceeded the maximum plasma levels recorded after a therapeutic dose of the drugs [10, 13, 14].

Results

MPZ showed nanomolar affinity for α_1, D_2 and histamine H_1 receptors, weak affinity for muscarinic cholinergic receptors and no detectable affinity for 5-HT_3 receptors (Table 2). Cisplatin, carboplatin, etoposide and doxorubicin had little or no detectable affinity for the receptors tested. Vincristine had nanomolar affinity for muscarinic cholinergic receptors but no detectable affinity for any of the other receptors (Table 2).

Discussion

Chemotherapy-induced emesis may be mediated by a combination of peripheral and central pathways. This study shows that the proposed central emetic effect of chemotherapeutic agents is not caused by a direct interaction with brain dopamine D_2, 5-HT_3, muscarinic cholinergic, histamine H_1 or α_1-adrenergic receptors. Vincristine

Table 2. K_i values obtained by the formula $K_i = IC_{50}/(1 + s/Kd)$, where s is the ligand concentration (see Table 1) and K_d is the dissociation constant: $D_2 = 0.063$ nM, 5-$HT_3 = 1.2$ nM, $H_1 = 2.4$ nM, MC = 0.014 nM and $\alpha_1 = 0.17$ nM

Drug	K_i values (nM)				
	D_2	5-HT_3	H_1	MC	α_1
DOX	>1100	>5500	>5500	650	>4000
VP-16	>1100	>5500	>5500	1500	>4000
JM8	>1100	>5500	>5500	>1000	>4000
VCR	>1100	>5500	>5500	74	>4000
CIS	>100	>550	>5500	>1000	>650
MPZ	0.68	>5500	18	1800	0.45

DOX, Doxorubicin; VP-16, etoposide; JM8, carboplatin; VCR, vincristine; CIS, cisplatin; MPZ, metopimazine; MC, muscarine cholinergic

showed binding affinity for muscarinic cholinergic receptors. An antagonist effect at this receptor is in concordance with the clinically well-known constipation associated with this drug. Our findings are in concordance with the results reported by Peroutka [34], who investigated the interaction of various chemotherapeutic agents with histamine H_1, dopamine D_2 and muscarinic cholinergic receptors. He found no detectable interaction with any of these receptors, but binding at 5-HT_3 receptors was not investigated [34]. These data do not exclude a central action on the receptors investigated, but the effect must be indirect, e.g. via release of a transmitter, for which dopamine and 5-HT have been suggested.

Ison and Peroutka [21] have demonstrated that neuroreceptor binding studies are of value in predicting antiemetic efficacy and side effects. They found an affinity for the dopamine D_2 receptor of 6.8, 21 and 160 (K_i in nM) for prochlorperazine, chlorpromazine and metoclopramide, respectively. In our study, MPZ had no clinically relevant affinity for the 5-HT_3 receptor. Thus, its antiemetic effect seems to be correlated to its high affinity for dopamine D_2 receptors, with a possible contribution arising from binding at histamine H_1 and α_1-adrenergic receptors (Table 2). MPZ had a K_i value of 0.68 nM for the dopamine D_2 receptor. This finding is in concordance with the results of clinical studies in which MPZ has been demonstrated to be more potent than chlorpromazine [43]. Metoclopramide is even less potent at the D_2 receptor [21], which could explain why in earlier clinical studies it showed no antiemetic effect when given orally at doses of 10–20 mg × 3 [30, 31]. The dose-limiting adverse effect of MPZ is orthostatic hypotension, which can be explained by the drug's high affinity for the α_1-adrenergic receptor (K_i value, 0.45 nM; Table 2).

The finding that the dopamine D_2 antagonist metoclopramide, when given at high doses, probably reduces cisplatin-induced emesis by antagonizing 5-HT_3 receptors [29] has led to the development of more selective 5-HT_3 receptor antagonists such as ondansetron, granisetron and tropisetron. The substantial antiemetic effect of these drugs has brought into question the role of dopamine receptor antagonists as antiemetics [45] and changed the focus from dopamine to serotonin as a neurotransmitter in emesis. Cisplatin increases the release of serotonin from enterochromaffin cells in the small intestine [9], but the release of serotonin cannot explain all kinds of emesis, as 5-HT_3 antagonists are not effective against emesis induced by apomorphine [29], copper sulphate, histamine [41] or motion sickness [42]. Furthermore, specific dopamine antagonists are known to produce only a moderate antiemetic effect when given at conventional doses. At high doses, however, MPZ and other antiemetics with antidopaminergic but without antiserotonin properties exert a significant antiemetic effect [6, 16, 22]. That serotonin release and action on 5-HT_3 receptors is not the sole mechanism involved in chemotherapy-induced emesis is also supported by data obtained in clinical trials in which the antiemetic effect of ondansetron has been enhanced by the addition of dexamethasone [36, 39] or MPZ [16].

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