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COMMENTARY

Role of 5-Hydroxytryptamine$_3$ (5-HT$_3$) Antagonists in the Prevention of Emesis Caused by Anticancer Therapy

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ABSTRACT. Most anticancer drugs are cytotoxic and produce various side-effects, among which nausea and vomiting are almost ubiquitous and usually extremely distressing to the patient. Cancer chemotherapy elicits two main phases of vomiting: an intense, acute phase of vomiting that occurs almost immediately following anticancer therapy and a milder, delayed phase of nausea and vomiting of longer duration. The mechanisms underlying the induction of nausea and vomiting after cancer chemotherapy are poorly understood but may be mediated by serotonin (5-hydroxytryptamine or 5-HT), particularly in the acute phase. Serotonin activates 5-HT$_3$ receptors, which function as ligand-gated ion channels located either in the periphery and/or in the central nervous system to produce emesis, among other effects. The peripheral 5-HT$_3$ receptors may be pharmacologically distinct from the central 5-HT$_3$ receptors and may exhibit some association with GTP-binding proteins. In addition, different populations may exist as distinct subtypes of the same receptor. The 5-HT$_3$ receptor antagonist ondansetron (GR 38032F) is effective in preventing the emesis induced by cytotoxic agents currently used in the treatment of many forms of cancer. Ondansetron has, comparatively, a much higher efficacy in the treatment of acute emesis following cancer chemotherapy than it does in the delayed phase, suggesting that the late phase of emesis may be mediated by other distinct mechanisms. BIOCHEM PHARMACOL 52;5:685–692, 1996.

KEY WORDS. chemotherapy; nausea; emesis; 5-HT$_3$ receptors; 5-HT$_3$ receptor antagonists; signal transduction

5-HT§ was first identified as the substance released from platelets during blood clotting. This substance was also able to stimulate smooth muscle, acting as a vasoconstrictor factor—hence the name "serotonin," derived from the regulation of "serum tone." In addition, a large amount of a smooth muscle contracting substance, named "enteramine," was found in the enterochromaffin cell system of the GI tract. This was subsequently found to be identical with serotonin. Serotonin was later found to be present in the CNS, although only a small percentage of total body 5-HT is of CNS origin, the majority being present in the GI tract.

Many transmitters have multiple, distinct receptor subtypes creating diversity and increasing the information-handling capacity of various cell types, including neurons [1]. Serotonin is no exception to this rule, and many receptor subtypes have been identified [2], with 5-HT$_1$ through 5-HT$_7$ (and subtypes of these) currently being recognized. Serotonin serves diverse physiologic roles, including modulation of nociception, and contraction and relaxation of smooth muscles in the airways, some blood vessels, and the GI tract. In the CNS, 5-HT serves as a neurotransmitter, modulating nociception, regulating sleep/wake cycles and other aspects of circadian rhythms, as well as eating behavior and mood, and influencing aggression, anxiety, and depression. 5-HT is also implicated in migraine and in cancer therapy-induced emesis [3]. Each of these functions can be attributed to interactions with specified 5-HT receptor subtypes. The development of selective agonists and antagonists for the various receptors has led to wide and expanding clinical uses of these drugs. It is the role of 5-HT and of the moderate affinity 5-HT$_3$ receptor subtype in anticancer-induced emesis that forms the focus of this review.

5-HT$_3$ receptors are located both centrally and peripherally, with particularly high concentrations being found in

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§ *Abbreviations:* 5-HT, 5-hydroxytryptamine, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; 2-methyl-5-HT, 2-methylserotonin; AP, area postrema; cAMP, adenosine 3′,5′-cyclic monophosphate; cGMP, guanosine 3′,5′-cyclic monophosphate; CTZ, chemoreceptor trigger zone; G-proteins, GTP binding proteins; GABA, γ-aminobutyric acid; GI, gastrointestinal; GTPγS, guanosine 5′-O-(3-thiotriphosphate); NCB-20 cells, neuroblastoma × Chinese hamster brain cell line; NG108–15 cells, neuroblastoma × rat glioma hybridoma cell line; N1E-115, neuroblastoma cell line; NO, nitric oxide; NTS, nucleus tractus solitarius; and PI, phosphatidylinositol.

the GI tract. 5-HT_3 antagonist agents continue to be evaluated for therapeutic use in the management of a variety of conditions, including anticancer therapy-induced emesis, GI motility disturbances, and carcinoid syndrome, and of migraine, anxiety, and schizophrenia. In the CNS, relatively low concentrations of 5-HT_3 receptors are present in the forebrain (especially the entorhinal area), amygdala, hippocampus, nucleus accumbens, and olfactory tubercle, with still lower concentrations present in the thalamus, striatum, hypothalamus, and other structures. By contrast, nuclei of the vagus and trigeminal nerves, tractus solitarius, and the area postrema have a relatively high density of 5-HT_3 receptors. Subtle pharmacological differences between these receptors and those at other sites have led to the suggestion of different 5-HT_3 subtypes, referred to as 5-HT_{3A} (vagus nerve, nodose and superior cervical ganglia, sensory nerves), 5-HT_{3B} (sympathetic and parasympathetic nerves), and 5-HT_{3C} (enteric nerves), although such classification is unclear [4].

The aim of this paper is to discuss the role of 5-HT_3 receptors in mediating the emesis induced by anticancer therapy [5] and its subsequent prevention by specific 5-HT_3 receptor antagonists. Emphasis will be given to the specificity of prevention of emesis by 5-HT_3 antagonists in the early phases of nausea and vomiting induced by cytotoxic chemotherapy or by radiation therapy.

PHYSIOLOGY OF EMESIS

Vomiting and nausea are natural protective reflexes designed to eliminate toxins from the GI tract and to prevent further ingestion of substances containing the same. It is important that the nausea and vomiting associated with chemotherapy be controlled effectively. Not only is it distressful and unpleasant to the patient, but it may also cause dehydration and impose mental and physical suffering that substantially diminish the quality of life. Nausea and vomiting can also compromise patient compliance, hence limiting the doses of radiation or cytotoxic drugs that can be used in the management of these patients.

The vomiting reflex is triggered by stimulation of chemoreceptors in the upper GI tract and mechanoreceptors in the wall of the GI tract which are activated by both contraction and distension of the gut as well as by physical damage [3]. Upon activation, the vagal afferents relay information to the central CTZ in the AP. The CTZ can also detect circulating toxins directly from the blood and the cerebrospinal fluid. Vagal afferents from the liver may also play a role in the relay of information to the CTZ. The vomiting reflex can also be stimulated through the vestibular labyrinthine system. The motor components of this reflex are integrated by the vomiting center in the brainstem, with output coordinated to produce vomiting [3].

DISTRIBUTION AND CHARACTERIZATION OF 5-HT_3 RECEPTORS

The 5-HT_3 receptor is synonymous with the classically described M-receptor of Gaddum and Picarelli [6]. In contrast to the remainder of identified 5-HT receptor subtypes, the 5-HT_3 receptor belongs to a superfamily of ligand-gated ion channels [7–9]. A cDNA clone encoding one subunit of the 5-HT_3 receptor (5-HT_3R-A) was isolated from NCB-20 cells [8]. The cloned 5-HT_3 receptor subunit shows structural similarity to the nicotinic acetylcholine receptor α-subunit (27% identity), $GABA_A$, *N*-methyl-D-aspartate (NMDA) and the strychnine-sensitive glycine (22% identity) receptors [8]. The receptor protein corresponds to a 487 amino acid sequence, with a topological organization consisting of four transmembrane-spanning domains (M1–M4). Consistent with the characteristics of other ligand-gated ion channels, both the NH_2 and carboxy terminals are located in the extracellular domain, with a long cytoplasmic loop connecting the M3 and M4 regions. The extracellular NH_2 terminal contains a Cys-Cys disulfide bridge, another characteristic feature of the superfamily of ligand-gated ion channels [8]. This loop region is highly conserved, exhibiting ~50% identity with those of the nicotinic and glycine receptors. The ligand-gated ion channels are generally pentameric structures consisting of 2–5 different subunits. The 5-HT_3 receptor is apparently no exception, with the cloned 5-HT_3 R-A having a molecular mass of ~56 kDa [8], and the entire receptor having a macromolecular size of ~259 kDa [10]. Thus, it is likely that the receptor exhibits a stoichiometry of at least two of the cloned 5-HT_3 R-A subunits with an additional three subunits that may be different and confer some of the tissue-specific differences that have been observed. Different studies have arrived at several estimates for subunit sizes ranging from 35 to 56 kDa [11].

Identification, characterization, and localization of 5-HT_3 receptors has been facilitated by the development of highly potent and selective drugs that bind to this receptor subtype [12]. The 5-HT_3 receptors were originally described in the periphery where the selective agonist 2-methyl-5-HT evoked a depolarization of neurons of the superior cervical ganglion [13], the vagus nerve [14], and of enteric neurons [15]. These receptors were later shown to be present in the central nervous system as well. Solubilization of 5-HT_3 receptor sites from membranes prepared from rat cerebral cortex and hippocampus allowed identification of binding sites using the radioligand [^{3}H]ICS 205–930, a potent and selective 5-HT_3 receptor antagonist [10, 14]. Radioligand binding studies using specific 5-HT_3 receptor antagonists have revealed the presence of a high density of 5-HT_3 receptors in areas known to be involved in the emetic reflex. Thus, there are 5-HT_3 receptors located on the vagal afferent terminals innervating the GI tract mucosa and also presynaptically on the same vagal afferent nerves located in the brainstem centers involved in the control of vomiting. These include the dorsovagal nucleus, NTS, and AP in several species, including humans [16–18].

5-HT_3 receptors have been demonstrated in cell cultures using [^{3}H]ICS 205–930 or the comparably potent and selective 5-HT_3 receptor antagonist [^{3}H]zacopride as ligands

[19, 20]. The most commonly used cell lines have been neuronal cell lines such as the mouse NG108–15 [21], as well as N1E–115 [22] and NCB-20 [23] cells. The presence of these receptors in such cell lines has permitted further biochemical, pharmacological, and electrophysiological characterization of the receptor due to the relative ease of appropriate experimentation with such model cell lines. The mRNA encoding the cloned 5-HT_3 receptor (5-HT_3 R-A) was identified in brain, spinal cord, and heart, but was absent in most peripheral tissues, such as the liver, spleen, or intestine [8]. This perhaps suggests the presence of different 5-HT_3 receptor subtypes to be found in the periphery as opposed to tissue of the central nervous system [4]. Similarly, different responses of 5-HT_3 receptors during differentiation and ontogeny have been demonstrated [24]. This suggests that alternative splice variants or subunit substitution may occur under some conditions.

ELECTROPHYSIOLOGY OF 5-HT_3 RECEPTORS

Derkach *et al.* [7] were the first to report that the 5-HT_3 receptor is a ligand-gated ion channel from their studies using single current recordings from outside-out patches of guinea pig submucosal plexus neurons. More recent electrophysiological studies in cultured cell lines have also shown that 5-HT produces transient electrical responses that can be blocked by selective 5-HT_3 receptor antagonists [4, 23, 25–27].

Activation of the intermediate affinity (~22 nM) 5-HT_3 receptors induces fast depolarizing responses that are usually followed by a rapid desensitization. These responses are mainly due to a permeability to monovalent cations, i.e. Na^+ influx and K^+ efflux, with a physiologic reversal potential near 0 mV [4]. The relative permeability to K^+ and Na^+ (P_K/P_{Na}) appears to fall into two main groups in different studies, one near a P_K/P_{Na} of 0.4 and one near a P_K/P_{Na} of 1.0, although rather widely ranging values have been obtained in similar preparations and by different investigators. Although cell culture conditions or recording techniques may be at least partly responsible for the discordant data, the existence of distinct 5-HT_3 receptor subtypes may also be postulated [4]. Large organic cations (e.g. ammonium, choline, and Tris) can also pass through the 5-HT_3 receptor channel [25], but divalent cations such as Ca^{2+} and Mg^{2+} are relatively excluded [25, 26]. However, under physiologic conditions, intracellular Ca^{2+} is increased by 5-HT_3 receptor activation [27]. This may be due to permeability of the receptor to Ca^{2+}, activation of voltage-dependent Ca^{2+}-channels due to 5-HT_3 receptor-mediated depolarization, or release of stored intracellular Ca^{2+} pools [25–27]. Although the physiologically important cation, Ca^{2+}, may not be directly involved in 5-HT_3 signal transduction, Ca^{2+} may co-regulate or modulate 5-HT_3 receptor-mediated responses to 5-HT and may decrease 5-HT_3 receptor affinity and rates of desensitization [4, 23, 24, 26]. Anions such as Cl^- appear to be only slightly permeant, if at all [25, 26]. Thus, the 5-HT_3 receptor is a cation-specific but relatively non-selective ion channel with a water-filled pore of approximately 7.6 Å minimum diameter [25]. Measurement of Hill coefficient values significantly greater than 1 indicates co-operativity, requiring the binding of two 5-HT molecules to the receptor before channel opening can occur [26].

It is unclear as to whether binding of 5-HT to the 5-HT_3 receptor site responsible for channel opening is also responsible for the rapid, reversible, desensitization observed in most studies—a different site having been suggested by some studies [25, 26]. Single-channel recordings in various preparations have suggested at least two discrete channels with high (15 pS) and low (9 pS) conductances [7]. During continuous exposure to 5-HT, the low-conductance channel appeared to desensitize, whereas the open probability of the high-conductance channel does not change [7]. In other preparations, remarkably small conductance values of 0.6 to 4 pS have been obtained [25, 26]. Such disparate values again suggest the likelihood of more than one receptor subtype and/or a variety of channel-operating mechanisms.

SIGNAL TRANSDUCTION AT 5-HT_3 RECEPTORS

Other than the ion channel mechanisms discussed above, little more is known about the signal transduction processes that may occur upon stimulation of 5-HT_3 receptors. There is evidence suggesting the production of various second messengers upon activation of central 5-HT_3 receptors, but the relevance of these observations remains to be clarified. However, the cloned neuronal cell lines discussed above have provided model systems for the study of signal transduction processes associated with these ion channels. In one early study, 5-HT was found to stimulate the production of cAMP in a concentration-dependent manner in primary cell cultures of colliculus neurons, suggesting a positive coupling of 5-HT_3 receptors present in these neurons to adenylate cyclase [28]. In a similar study, activation of 5-HT_3 receptors with 2-methyl-5-HT stimulated the production of cGMP in NG108–15 cells [29], with this effect being blocked by specific 5-HT_3 receptor antagonists such as ondansetron [29]. It seems that coupling of 5-HT_3 receptors with second messenger systems effected via G-proteins does not occur since pertussis toxin does not alter 5-HT_3-mediated responses and such responses continue to occur in nucleotide free bathing solutions after many hours [7]. In addition, intracellular dialysis with G-protein activators, such as CsF, AlF_4^-, and GTPγS, do not alter responses to 5-HT_3 receptor activation [25, 30].

Although G-protein signal transduction mechanisms are probably not directly involved in 5-HT_3 receptor signal transduction, modulation of responses by intracellular second messengers and phosphorylation seems probable. Thus, cAMP may modulate 5-HT_3 responses since rates of desensitization are accelerated by forskolin, an adenylate cyclase

activator [30]. Similarly, the phosphodiesterase inhibitor isobutylmethylxanthine (IBMX), the cAMP analogue dibutyryl cAMP, the G_S-protein activator cholera toxin, and prostaglandin E_1 all increase intracellular cAMP concentrations and accelerate the rate of 5-HT_3 receptor desensitization [25, 30]. Conversely, intracellular dialysis of recorded cells with the non-hydrolyzable ATP analogues AMP-PNP (5′-adenylylimidodiphosphate) and AMP-PCP [adenylyl(β,γ-methylene)diphosphate] both decelerate the rate of desensitization of 5-HT_3 receptor-mediated responses [30]. Although it appears that these changes in desensitization appear to be related and due to the same mechanism, this is probably not the case although it is beyond the scope of this paper to discuss this further [see Ref. 26 for further details]. In addition to at least two distinct roles for cAMP and the likely associated alterations in protein phosphorylation, inhibition of protein kinase C by the potent but non-specific inhibitor staurosporine reversibly reduces the amplitude of 5-HT_3-mediated responses [31]. Again, phosphorylation of a 5-HT_3 receptor protein subunit or associated regulatory protein is suggested, but further extensive biochemical studies will be required to clarify this point.

5-HT_3 receptor agonists have been shown to suppress neuronal firing and to stimulate PI formation by direct interaction with 5-HT_3 receptors in rats. In rat fronto-cingulate and entorhinal cortical slices, microiontophoresis of the 5-HT_3 agonists phenylbiguanide and 2-methyl-5-HT produces increases in PI turnover and suppressed firing of medial prefrontal cortical cells [32, 33]. This action may be blocked by the selective 5-HT_3 antagonists granisetron, ICS 205–930, ondansetron and (±)-zacopride. Phosphoinositide hydrolysis may be one of the transducing mechanisms for 5-HT_3 receptors in the fronto-cingulate and entorhinal cortices of rats. This suggests that some 5-HT_3 receptors in the brain may be coupled to second messengers via G-proteins, whereas in the periphery, they are more usually present as direct ligand-gated ion channels [7]. However, more thorough study is needed to evaluate the relevance of these differences. Perhaps in some systems, particularly in the periphery, these receptors are needed to convey fast sensory inputs, whereas in the brain they may (also) serve for slower processing of information.

Although Ca^{2+} may not be directly involved in 5-HT_3 receptor-mediated signal transduction, there is, as discussed above, substantial evidence that this important regulatory cation is involved in several aspects of such signal transduction [4, 23–27]. Since Ca^{2+} is critical for a variety of regulatory processes, including cellular polarization events, protein phosphorylation, transmitter release, and all other subcellular motile processes, it seems likely that this ion is either directly or indirectly involved in both short- and long-term responses mediated by 5-HT_3 receptors. A series of elegant studies by Reiser and colleagues indicates that stimulation of 5-HT_3 receptors causes a fast depolarization that leads to an increase in intracellular Na^+ and Ca^{2+} and a rise in intracellular Ca^{2+} activity [27]. This increase in intracellular Ca^{2+} activates NO synthase to increase formation of NO from L-arginine, which is able to stimulate guanylate cyclase and increase intracellular cGMP concentrations [29, 34]. Such increases in NO and cGMP may exert both immediate and long-term effects on numerous biochemical events in the local environment.

MECHANISMS BY WHICH CANCER CHEMOTHERAPEUTIC AGENTS INDUCE EMESIS

Treatment with anticancer drugs, such as cisplatin, or radiotherapy for cancer patients causes severe nausea and emesis. Cisplatin is an active cytostatic platinum-based agent, and cancer chemotherapy with this drug is one of the most emetic cytotoxic treatments known [35]. Most studies have used cisplatin as the anticancer agent of choice in their models for induction of emesis. In the absence of effective antiemetic protection, chemotherapy based on high-dose cisplatin induces vomiting in almost all patients. The acute phase of vomiting begins 2–3 hr after chemotherapy and lasts for about 8 hr following cisplatin administration, although the time course varies somewhat with different drugs [36]. A milder phase of nausea and vomiting (delayed emesis) then develops which may last for 3–5 days [37].

There are several limitations to the use of various antiemetic agents such as some of the antihistamines and dopamine agonists, e.g. apomorphine, for treatment of anticancer therapy-associated emesis. These include dose-limiting effects of centrally acting dopamine antagonists due to their undesirable side-effects, e.g. extrapyramidal side-effects [3, 38]. Such limitations have encouraged the search for better antiemetic drugs. In recent years, this has led to the development of the anti-serotonergic agents.

The mechanisms by which cancer chemotherapeutic agents induce emesis are not well understood, and several theories have been postulated. Direct stimulation of the CTZ and/or the vomiting center by the anticancer drugs has been postulated. However, this theory has been put to question because of the relatively long latency to onset of vomiting. Certainly the CTZ is involved, and electrolytic lesions confined to the AP abolish both radiation- and apomorphine-induced vomiting in the dog, but this response seems to be species specific [39]. Furthermore, vagotomy and sympathectomy have been shown to abolish cisplatin-induced emesis in the ferret [40], suggesting the involvement of peripheral inputs possibly from the GI tract and/or other visceral organs. However, a mechanism implicating direct activation of the CTZ by the toxins may still hold true in the case of delayed emesis. Based on the findings that peripheral factors may be important mediators of emesis produced by cancer chemotherapy, a humoral model of induction of emesis was then proposed [41]. This model suggested that endogenous factors, such as 5-HT, formed or released after radiation or cancer chemotherapy, stimulate

the CTZ via the circulation. However, in view of a variety of inconsistencies, this model did not receive much support either [41].

A perhaps more acceptable model proposes that neural inputs from the vagus and sympathetic fibers of the GI tract are important in the mediation of emesis [41]. Indeed, sectioning of both of these inputs prevented cytotoxic drug-induced emesis in the ferret [40]. Since most anticancer drugs produce nausea and vomiting, the mechanism is likely to be common to most such anticancer drugs. The common mediator is thought to be 5-HT and the mode of action is probably local, involving activation of afferent neural fiber terminals present in the abdominal viscera [41, 42]. Thus, it is supposed that upon stimulation by cytotoxic drugs, 5-HT is released from enterochromaffin cells of the GI tract mucosa which then activates presynaptic vagal afferents and stimulates the emetic reflex. Serotonin may also be released following damage to the GI tract mucosa. Studies in the ferret have shown that cisplatin produces severe mucosal damage to the ileum and jejunum and that severity of the emesis is related to the extent of the damage [41, 43]. Cisplatin may also act by increasing acetylcholine release, which, acting via muscarinic receptors, stimulates 5-HT release from the enterochromaffin cells. This explains the action of the cholinergic blockers scopolamine and hexamethonium in reducing the response [41].

Cisplatin-induced emesis in humans can be antagonized by pretreatment with the 5-HT synthesis inhibitor chlorophenylalanine [44]. While there has been a demonstration of increased urinary excretion of the serotonin metabolite 5-HIAA in humans following cancer chemotherapy [37], another study in humans indicated that there is no increased release of 5-HT during the delayed phase of emesis [45]. As discussed above, electrophysiological studies have shown that 5-HT can activate abdominal vagal afferents and that this response can be blocked by antiemetic doses of the 5-HT_3 receptor antagonist ondansetron [46]. Suggestions of presynaptic inhibition or facilitation by 5-HT_3 receptors of the release of neurotransmitters including but not limited to 5-HT indicate that such mechanisms may also be involved in various components of the emetic response during anticancer therapy.

The 5-HT_3 receptor antagonist ondansetron injected directly into the AP in the ferret causes a dose-related inhibition of vomiting and retching [47]. It may seem unlikely that serotonin released from enterochromaffin cells circulates to mediate a central effect since 5-HT is metabolized rapidly. The ventral surface of the AP contains neurons that secrete serotonin, and hence the possibility of a direct action of activation of 5-HT_3 receptors located presynaptically on terminals of the vagus nerve within the vomiting system exists [42]. Unilateral/bilateral vagotomy in ferrets leads to a decrease in the number of 5-HT_3 receptors in the brainstem [48], indicating a possible role of central 5-HT_3 receptors in emesis. In contrast, injection of the 5-HT_3 receptor agonist 2-methyl-5-HT into the AP of ferrets induces only mild retching [48]. In relation to this finding in the ferret is that binding of 5-HT_3 receptor ligands is low in the AP as compared with that found in the NTS [18]. 5-HT_3 receptor antagonists have been shown to have antagonistic activity at receptors on the vagal afferents terminating in the NTS [49]. Although the role of central 5-HT_3 receptors in the induction of acute emesis is not certain, it seems probable that both central and peripheral mechanisms are involved in causing such emesis.

More studies are needed with improved methods to clarify the exact mechanisms involved in the emesis induced by anticancer agents. Some of the studies discussed in this article have substantial drawbacks. For example, measurement of urinary 5-HIAA may be a poor index of 5-HT activity affecting 5-HT_3 receptor sites. The ferret is widely accepted as a good model for studying mechanisms of emesis and its prevention; however, this may not be the best model, and it would be interesting to compare the effects in the different species sometimes used for such studies, e.g. dogs, cats, and humans. There is also the possibility that either a subtype of the 5-HT_3 receptor [4] or another 5-HT receptor is involved in the emetic response. Indeed, 5-HT_4 receptors have been shown to participate in copper sulfate-induced emesis [50]. In relation to this, many 5-HT_3 antagonists also exhibit a high affinity at 5-HT_4 receptors. 5-HT_3 antagonists also lack the ability to prevent other kinds of vomiting and only appear to be specific for emesis induced by anticancer drugs and radiotherapy, e.g. 5-HT_3 antagonists fail to prevent the vomiting associated with motion sickness or following administration of xylazine [51], or the emesis induced by dopamine and opiate receptor agonists [52]. These data may argue against a role for 5-HT_3 antagonist activity in the vomiting center. It appears that the serotonin theory may only apply to the early phase of vomiting following anticancer therapy, and that only peripheral mechanisms are involved. Although the delayed emesis may be mild, it still remains a concern in the use of anticancer drugs especially because it may be of a chronic, persistent nature. Perhaps the mechanisms involved in the delayed emesis may be a result of direct actions of the toxins or their metabolites in the CTZ and may involve roles for other systems, such as the immune system. In view of the altered desensitization properties of 5-HT_3 receptors [25–27, 30], and potential alterations in receptor subunit composition, it is possible that the delayed emetic response may be due to altered responsiveness of either peripheral or central 5-HT_3 receptors in the later phases. Furthermore, it is clear that further studies are necessary to determine the specificity of the induction of nausea and vomiting by anticancer agents and what makes this kind of emesis different from other types.

CLINICAL USE OF 5-HT_3 ANTAGONISTS

The antiemetic effects of 5-HT_3 receptor antagonists may result from blockade of 5-HT-induced depolarization of the

generator region of the vagal afferents, thus preventing the generation of action potentials that provide the emetic signal to the CNS vomiting center. Although 5-HT_3 receptor antagonists are definitely effective against acute emesis following cancer therapy [53], there is evidence that they also partially antagonize emesis during the delayed phase [54]. Ondansetron, the first of these antagonists to be put to clinical use, is a selective antagonist of the 5-HT_3 receptor [55, 56]. Ondansetron is well tolerated by all age groups [57], and the pharmacological properties have been well described [58]. Ondansetron is structurally similar to 5-HT (it bears an indole nucleus) and is potent and safe, presenting no adverse effects on normal behavior or on cardiovascular parameters even at high doses [59]. It is important however, to make note of the most common side-effect associated with ondansetron therapy in humans, i.e. headache [56].

Antiemetic effects of ondansetron have been shown to increase when used in combination with dexamethasone [42]. Such observations support the suggestion for a role of, for example, immune system products in the induction of emesis following anticancer therapy. In relation to this, CNS 5-HT has been shown to exert both inhibitory and facilitatory roles in the regulation of hypothalamo-pituitary-adrenocortical secretion, with 5-HT_3 receptors mediating relatively mild stimulating effects [60]. It is perhaps of interest that the 5-HT_3 antagonist MDL-72222 was only effective in reducing adrenocortical responses to acoustic stimulation, not affecting responses to footshock, restraint stress, conditioned fear, or to injections of interleukin-1α or cocaine [61]. Since footshock, restraint stress, and conditioned fear all produce defecation in the rat, it is interesting that the 5-HT_3 antagonist had no effect on the associated adrenocortical response. This observation suggests either discrete central neural pathways regulating the two responses, or a lack of a role for central 5-HT_3 receptors in the regulation of GI tract motility.

Ondansetron antagonizes effects in both the CNS and the GI tract and has been approved by the Food and Drug Administration for the treatment of chemotherapy-induced emesis. Nevertheless, it may have effects on other channel or receptor functions independent of its 5-HT_3 receptor blocking effects. Ondansetron has been shown to have weak affinity for other receptors, e.g. μ-opiate binding sites [42]. In a recent study, ondansetron specifically blocked voltage gated potassium channels in TE671 human neuroblastoma cells [62], whereas a range of other 5-HT_3 receptor specific antagonists failed to produce the same effect. It is possible, therefore, that part of the beneficial effects of ondansetron in the prevention of emesis induced by anticancer therapy may be attributed to effects in addition to its specific 5-HT_3 antagonistic activity.

In addition to the rather successful introduction of ondansetron to clinical use, other 5-HT_3 receptor antagonists such as granisetron, zatosetron, and tropisetron are still in clinical testing [63]. The selective 5-HT uptake inhibitor litoxetine, currently under development as an antidepressant, has also been found to prevent cisplatin-induced emesis in the ferret [64]. Thus, it seems likely that other drugs acting to alter 5-HT transmission will be developed to control cancer therapy-induced emesis.

CONCLUSIONS

5-HT_3 receptors are present on vagal afferents in the GI tract mucosa and in the brainstem centers, regulating vomiting reflexes. Initiation of emesis is probably due to the release of serotonin from enterochromaffin cells in the small intestine which activates vagal afferent nerves via 5-HT_3 receptors. Delayed emesis may involve central 5-HT_3 receptors and/or serotonin stores in the enterochromaffin cells which may have been replenished, or it may not involve serotonin at all. Specific 5-HT_3 receptor antagonists such as ondansetron prevent emesis, probably by competitive inhibition at the 5-HT_3 receptor sites. The molecular mechanisms of action of 5-HT_3 receptor agonists at the cellular level need further investigation, especially in determining any differences of receptor subtypes and their roles in the periphery versus the central nervous system.

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