Evidence for a Re-Evaluation of the Neurochemical and Anatomical Bases of Chemotherapy-Induced Vomiting

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1. Introduction

Historically, emesis was considered a simple reflex localized to a putative "vomiting center" in the brain. However, it quickly became clear that this idea was insufficient, and that emesis was a much more complex behavior than first thought. This was exemplified by initial attempts to use early emesis research to manage vomiting caused by the highly emetogenic—but highly effective—chemotherapeutic agent cisplatin (chemotherapy-induced vomiting), which fell short of expectations. Some success (10−30% of patients were afforded complete protection) was eventually found through the antidopaminergic phenothiazine-based antiemetics, especially when combined with dexamethasone or related glucocorticoids,[1] but this was limited to the acute phase of chemotherapy-induced vomiting (vomiting immediately following cisplatin administration), and typically only two-thirds of patients responded even then.[2] Clinical research on phytocannabinoids as antiemetics also showed promise at this time, but little progress was made due to their problematic side effects.[3,4] Finally, a breakthrough was made with the development of the 5-HT_3 serotonin receptor antagonists

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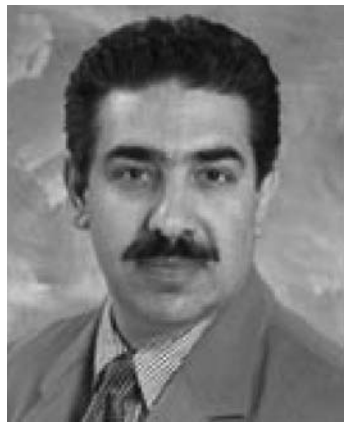

Nissar A. Darmani was born in Kabul, Afghanistan. In 1971, his family moved to Great Britain, where he completed his high school undergraduate and postgraduate studies. In 1979 he was awarded joint honors B.Sc. degree in biochemistry and pharmacology from the University of Leeds. Thereafter, he worked for 3 years at the immunohematology center, Cambridge. He obtained his M.Sc. degree in applied pharmacology from the University of Wales (UWIST) in 1984. In 1988 UWIST awarded his Ph.D. degree in Neuropharmacology. He pursued postdoctoral research on the neuropsychopharmacology of drugs of abuse at the department of Pharmacology, Medical College of Virginia. In 1991, Dr. Darmani accepted a Faculty position in the department of Pharmacology at the Kirksville College of Osteopathic Medicine, A.T. Still University of Health Sciences. He rapidly rose through the academic ranks and became tenured professor in 2000. In March 2005, Dr. Darmani accepted the leadership position as the Chair of Basic Medical Sciences at the College of Osteopathic Medicine of the Pacific (COMP), Western University of Health Sciences (WUHS), Pomona, California. In 2009 he was promoted to the Assistant Dean for Basic Sciences and Research at COMP. Dr. Darmani has several avenues of research interest that include: (1) developmental effects of drugs of abuse on the newborn, (2) serotonergic mechanisms of cocaine's actions, and (3) mode of action of antidepressant drugs. His latest NIH-funded research project involves the role of Δ^9-THC and synthetic cannabinoids on chemotherapy- and radiotherapy-induced vomiting. Indeed, Dr. Darmani's laboratory was the first research center to demonstrate the mechanisms of antiemetic actions of marijuana. Professor Darmani was honored with the Irvin M. Korr research award by the American Osteopathic Association at their 49th annual research conference in 2005. Dr. Darmani has been successful in obtaining several million dollars of research grants from numerous funding agencies including the pharmaceutical industry, the National Institute of Health, the Department of Defense, and the Environmental Protection Agency.

Andrew Ray was born in New Jersey and raised in Miami, Florida, then went on to get his B.S. with honors from Georgia Tech in 1991. He stayed in Atlanta throughout his graduate school education, getting his Master's in Science from Georgia State University in 1994, then finally a Ph.D. in Neuroscience from Emory University in 2001, where he studied the anatomical underpinnings of sleep−wake state control under the tutelage of David Rye. He then moved to California to begin postdoctoral work, joining the Center for Narcolepsy Research in the lab of Emmanuel Mignot and studying the expression of orexin/hypocretin in zebrafish. He continued his postdoctoral work with Steven Henriksen at The Scripps Research Institute in La Jolla, CA, in 2003, examining the form and behavioral function of novel connectivity between the ventral tegmental area of Tsai and the forebrain. By 2006 he had not only gotten married and become stepfather to three children, he had also landed at the Western University of Health Sciences lab of Nissar Darmani, where he has been studying the interplay of the brain and gut as it relates to vomiting in a unique animal model, the least shrew. His research interests include neuroanatomy, neurochemistry, and understanding how neural systems can create sleep−wake behaviors, emotional states, and consciousness, in the same way an orchestral piece is created from so many individual instruments.

(e.g., ondansetron and other "setrons"), which were significantly more efficacious, enough so to rapidly become the gold standard treatment and bring about the clinical demise of the phenothiazines and phytocannabinoids as first-line antiemetics against chemotherapy-induced vomiting.[5,6] Despite the setrons' antiemetic efficacy, chemotherapy-induced vomiting continues to present problems, as the delayed phase (a series of emetic bouts following a multiday quiescent period subsequent to the acute phase) is still only partially controlled. Continued research led to the hypothesis of the tachykinin substance P (SP) and its NK_1 neurokinin receptor, as potentially mediating the delayed phase of chemotherapy-induced vomiting.[7,8] This culminated in the development of aprepitant and other NK_1 receptor antagonists as putative antiemetics when combined with standard antiemetic regimens (i.e., a 5-HT_3 antagonist plus dexamethasone) for the delayed phase.[9,10] Even with the advent of these drugs, only up to 80% of patients can be completely protected from chemotherapy-induced vomiting. In this case, "completely" is defined as the prevention of all bouts of vomiting, whether acute or delayed phase. Furthermore, nausea appears to be mediated by at least some mechanisms that are different from emesis, and is even more poorly controlled than vomiting. These deficiencies and the extensive presence of cannabinoid CB_1 receptors in the emetic reflex arc circuitry and their interaction with serotonergic and tachykininergic systems have rekindled interest in cannabinoids as antiemetics, if only within research circles. As the clinical record demonstrates, chemotherapy-induced vomiting is far from a "simple reflex arc". Rather, expression of chemotherapy-induced vomiting is highly complex, requiring the stimulation or inhibition of several neurotransmitter systems, each acting on either one or multiple receptor subtypes, and on both central and peripheral nervous system components, all of which is within a tightly regulated temporal sequence. Further complicating matters, chemotherapy-induced vomiting also engages inflammatory cellular activity via leukotriene- and prostaglandin-related intercellular mediators. Indeed, the material covered in this review may be extensive but not necessarily exhaustive. Other peptides or cellular messengers may prove to be emetic mediators, and as yet unidentified signaling systems may even remain to be discovered by basic scientists. For example, chemotherapy-induced vomiting may involve free radical formation and/or inflammatory processes, both of which are also implicated in platin-related toxicity.[11−14] From the clinical standpoint, investigation must continue into the efficacy of various combinations of known antiemetics. Because it can be induced by so many diverse factors, chemotherapy-induced vomiting is the most reliable and easily reproducible side effect of cisplatin therapy and is, in fact, frequently reported by patients as both the most distressing side effect and the primary reason for prematurely dropping out of their chemotherapy.[15,16]

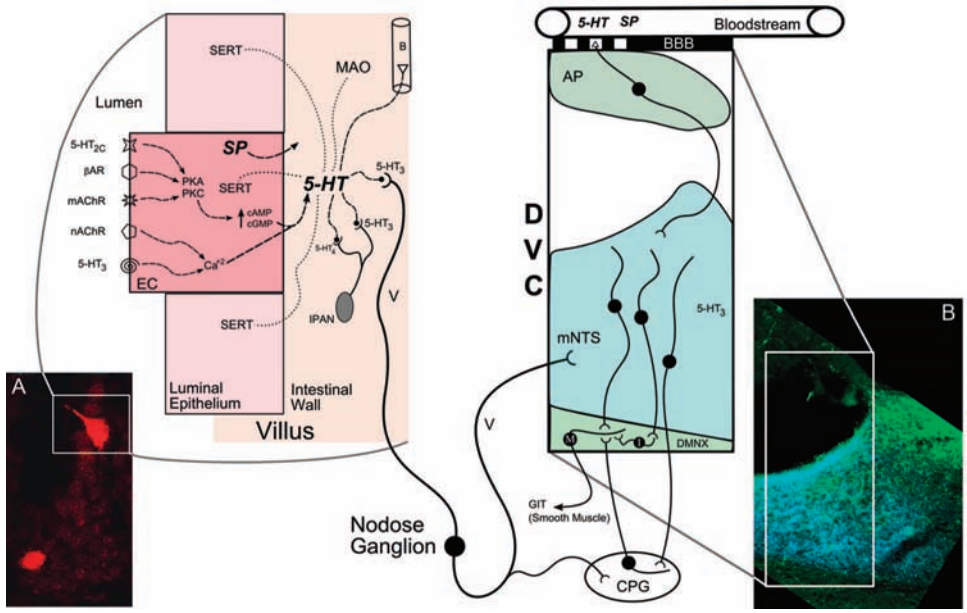

Figure 1. Key emesis-mediating components of the brain−gut axis. In chemotherapy-induced vomiting, the current tenets state that cisplatin and related chemotherapeutics induce acute vomiting by a powerful release of serotonin (5-HT) from enterochromaffin cells embedded in the luminal epithelium. Photomicrograph A depicts a strip of least shrew luminal epithelium (boxed area) from a villus immunolabeled for 5-HT (red) to highlight enterochromaffin cells. Enterochromaffin cells can also be stimulated to release 5-HT by a variety of luminal membrane-bound receptors, ultimately leading to stimulation of various second messenger systems and secretion of 5-HT (dashed lines in diagram). Secreted 5-HT can act locally via $5\text{-HT}_{3/4}$ receptors on vagal and intrinsic primary afferent neuron fibers in the intestinal wall, or may act distantly via the bloodstream to stimulate (1) the enteric nervous system and (2) possibly the dorsal vagal complex of the medulla. Likewise, substance P (SP) can be released by cisplatin: (1) from enterochromaffin cells where it either can bind locally to specific neurokinin NK_1 receptors in the gastrointestinal tract or on vagal afferents, or can diffuse into the bloodstream and enter the brainstem to induce vomiting; (2) from vagal afferent terminals in the brainstem to cause emesis. Photomicrograph B depicts a coronal hemisection of the dorsal vagal complex (boxed area) of the least shrew, immunolabeled for SP (blue) and 5-HT (green). Vagal afferents projecting from the nodose ganglion to both the gut and brain, and area postrema neurons accessing the bloodstream through the locally fenestrated blood−brain barrier, enable rapid communication between the brain and gut. Vagal stimulation of the nucleus of the solitary tract (or serotonergic and/or tachykininergic stimulation of the area postrema) induces the emetic motor output of gastrointestinal tract smooth muscle via action on both motoneurons (M) and interneurons (I) of the dorsal motor nucleus of the vagus, while concomitant stimulation of the central pattern generator area near the nucleus ambiguus coordinates related prodromal respiratory/salivatory activity (precursors to vomiting) with the actual act of vomiting. Abbreviations: 5-HT, serotonin; $5\text{-HT}_{\#}$, serotonin receptor subtype; AP, area postrema; βAR, beta-adrenergic receptor; B, blood vessel; BBB, blood−brain barrier; CPG, central pattern generator area; DMNX, dorsal motor nucleus of the vagus; DVC, dorsal vagal complex; EC, enterochromaffin cell; GIT, gastrointestinal tract; IPAN, intrinsic primary afferent neuron; mAChR, muscarinic cholinergic receptor; MAO, monoamine oxidase; mNTS, medial subnucleus, nucleus of the solitary tract; nAChR, nicotinic cholinergic receptor; PKA/PKC, protein kinase A/C; SERT, serotonin reuptake transporter; V, vagal afferent nerve fiber.

This review will focus on the multifaceted nature of chemotherapy-induced vomiting. In summarizing this complex system, we will describe: (1) the localization, metabolism, and release of the well-investigated emetic neurotransmitters such as dopamine, serotonin, and SP; (2) the localization, metabolism, and release of the lesser known eicosanoid and endocannabinoid-related transmitters; and (3) the specific receptor subtypes identified with, or suspected of involvement in, emesis. Because the expression of emesis is not localized to a single, centrally located "vomiting center" as was historically believed,[17] we will also describe the chemotherapy-induced vomiting-related modulatory effects of these emetic neurotransmitters on diverse components of the emetic reflex arc in the peripheral (vagal afferents, enteric nervous system, enteroendocrine cells) and central (dorsal vagal complex, central pattern generator) anatomical compartments. This information will finally be synthesized into a new hypothesis describing mechanisms of how chemotherapy-induced vomiting in general, and cisplatin in particular, specifically induce both phases of vomiting. This hypothesis will be a necessary revision to the currently accepted dogma of the neurotransmitter basis of chemotherapy-induced vomiting. The current tenets hold to a mechanism involving sequential activity of peripheral serotonin and central SP for induction of acute and delayed phases of chemotherapy-induced vomiting, respectively, and fail to take into account dopaminergic and other activity, neurochemical interactions between transmitters, and the combined activation of central and peripheral substrates, despite evidence for each of these features in the mechanism of chemotherapy-induced vomiting. This review will demonstrate the complexity of chemotherapy-induced vomiting, while presenting a revised hypothesis whose complexity matches that of the mechanism of chemotherapy-induced vomiting.

2. Anatomical Substrates of Emesis

2.1. Overview

The emetic reflex arc is highly complex and, despite extensive work, is only partially characterized. The reflex must be able to respond to a wide variety of toxic agents (e.g., chemotherapeutic drugs) or conditions (e.g., radiation, excessive motion), and the anatomical foundation of the arc has developed to meet this criterion. Figure 1 provides a visual reference and outlines the many key features of the anatomy of the emetic reflex arc. Emetogens can act directly in the gastrointestinal tract and/or indirectly by activating central nervous system (CNS) nuclei through stimulation of

vagal afferents whose somata are in the nodose ganglion. In the CNS, both the cluster of medullary nuclei described as the dorsal vagal complex and a more ventrolaterally localized group of cells that make up the central pattern generator are key sites in the mediation of emesis. Connecting the gastrointestinal tract and dorsal vagal complex/central pattern generator, the vagus afferent and efferent nerves provide for transferring information between gastrointestinal tract and brain. Not only can emetogenic signals be transmitted vagally, they can be transmitted humorally via the bloodstream by passing the blood−brain barrier and directly activating the dorsal vagal complex. Indeed, these events appear to be key initiating steps in chemotherapy-induced vomiting, the mechanism of which will be discussed more thoroughly later in this review. Motor output from other dorsal vagal complex neurons can then be coordinated to stop peristalsis and produce the giant retroperistaltic contraction inherent to emesis. The relevant anatomy of each compartment, both central and peripheral, will be discussed in more detail below.

2.2. Gastrointestinal Tract and Enteric Nervous System

In chemotherapy-induced vomiting and many other forms of vomiting, emetic signaling is initiated in the gastrointestinal tract. Critical to the emetic reflex, enterochromaffin cells (also called enteroendocrine cells) are epithelial cells in the intestinal mucosa that store serotonin (5-HT) and SP and act as sentinel cells. A range of receptor subtypes that bind a variety of neurotransmitters or other signaling molecules are present on enterochromaffin cells, allowing them a wide response profile (see Figure 1 for examples). When a toxin is absorbed from the lumen, or enterochromaffin cells exposed to it via intestinal microvasculature, the cells release vast amounts of 5-HT into the mucosal lining and the intestinal wall and thus into the bloodstream. Interestingly, it is still unclear how the intestinal lumen differentiates between ingested toxins and ingested food (and some might argue that depending on the restaurant, there *is* no difference). However, the presence of such a wide variety of receptors on enterochromaffin cells, and the exocytotic release of 5-HT subsequent to activation of these receptors as a common element of exposure to many different emetogens (reviewed by Minami et al.[18]), suggest that enterochromaffin cells are a primary mediator of toxin/food differentiation. It should be noted, though, that enteric mast cells may also release 5-HT and other inflammatory emetic mediators following exposure to a toxin. As mentioned, many of the receptors present on enterochromaffin cells, including adrenergic, cholinergic, and serotonergic receptors, induce release of 5-HT. Others, however, including tachykininergic and histaminergic receptors, can inhibit release.[19,20] Interestingly, enterochromaffin cells (or mast cells) may be stimulated to release 5-HT by prostanoids, and especially by chemotherapeutics that induce vomiting, such as cisplatin.[18,21] Primary afferent neurons within the enteric nerve plexi demonstrate an enhanced excitability and increased firing rate upon exposure to 5-HT, mediated by 5-HT_3 and possibly 5-HT_4 receptors.[22−24] The enteric nervous system (ENS) is larger and more complex than other components of the peripheral nervous system, which reflects its ability to regulate enteric functions in the absence of CNS input.[25] The enteric plexi contain intrinsic primary afferent neurons and interneurons that enable the ENS to independently mediate integrative responses to local stimuli. Many of the small- and large-molecule emetic neurotransmitters involved in chemotherapy-induced vomiting that are found in the CNS have also been identified in the ENS. These include 5-HT,[26] dopamine[27] and SP.[28] Notably, all three of these neurotransmitter systems, and some of the emetogenic eicosanoids described in section 7, have been shown to activate vagal afferents terminating in the intestinal wall—a key commonality of emetic reflex induction.[18]

Emetogenic neurotransmitters released into the intestinal wall or in the bloodstream act on corresponding specific receptors found in the enteric nerve plexi and on intestinal smooth muscle to modulate contractility and rhythmicity. Neurons within the submucosal and myenteric nerve plexi are arranged in ganglia-like clusters, with extensive interconnecting fibers between clusters. Each nerve plexus innervates different layers of the intestinal wall, and the neurons themselves use a variety of neurotransmitter systems, including nitrergic, cholinergic, tachykininergic, and vasoactive intestinal peptidergic (VIPergic) systems.[29] In the myenteric plexus, interneuron clusters connect longitudinally to interneurons in adjacent clusters, as well as with periodically dispersed motoneurons. These motoneurons, both inhibitory and excitatory, innervate the longitudinal and circular smooth muscles that are the contractile effectors for the various peristaltic motions of the intestine. The submucosal neuron clusters are primarily cholinergic, tachykininergic, and/or VIPergic, but immunohistochemical evidence suggests numerous other peptide signaling molecules are present, including but not limited to galanin, neuropeptide Y, and dynorphin.[30−32] The neurons are functionally grouped into interneurons, secretomotor neurons, and sensory neurons, depending on transmitter/peptide content and which layers of intestinal wall are innervated. In general, the submucosal plexus functions to mediate mucosal secretion and to couple it with myenteric plexus-mediated peristaltic activity.

Specialized neurons within both nerve plexi, intrinsic primary afferent neurons (IPANs), seem to be the key integrative neurons within the ENS and coordinate both the contraction and relaxation phases of peristalsis. IPANs also appear to be critical to the generation of the retroperistaltic contraction used in vomiting to push the toxic gastrointestinal tract contents back into the stomach for expulsion. Finally, IPANs in the submucosal plexus directly innervate ascending interneurons in the myenteric plexus, whereas IPANs in the myenteric plexus directly innervate the mucosa (and, thus, enterochromaffin cells), making these neurons critical to the coupling of activity across the different layers of intestinal wall.[33,34] A second set of specialized cells that mediate gut motility are the Interstitial Cells of Cajal (ICC). ICC appear to be hybrid neuron-muscle cells and generate intrinsic pacemaker activity, which provides timed waves of slow, depolarizing electrical activity to the gut wall. This activity by itself does not cause increased neuronal firing, but in concert with other stimuli (e.g., vagal afferent activity) is responsible for increased neuronal firing during peristaltic wave activity.[35]

While the importance of 5-HT release by enterochromaffin cells in generating emesis cannot be understated, other neurotransmitter systems have also been found to be potent modulators of the emetic reflex in the periphery. The nerve plexi of the ENS have been found to contain many SP-containing neurons, with fibers innervating the mucosa extensively.[28] NK_1 and possibly NK_3 receptor-mediated

substance Pergic neurotransmission through these fibers potently excites ENS neurons and contracts GI smooth muscle.[36−39]

2.3. Vagus Nerve: The Bridge between Brain and Gastrointestinal Tract

The 10th cranial nerve (X), the vagus, mediates autonomic information transfer between the gastrointestinal tract and the brain. A mixed nerve, somata for GI-related vagal afferents are found in the nodose ganglion near the jugular vein, whereas somata for vagal motoneurons are located in the dorsal motor nucleus of the vagus (DMNX, see below). Although the ENS is a fully functional local "nervous system" in the gastrointestinal tract even when dissociated from the CNS, the emetic reflex arc is nearly abolished following bilateral vagotomy. The remaining emetic activity can be abolished if the splanchnic nerve is lesioned in addition to both vagi.[40−42] Nodose neurons project extensively branched afferent fibers in both ascending and descending directions, such that the same neurons innervate both the medulla and a segment within the ENS. The vagal afferents are glutamatergic and appear to corelease SP, thus providing excitatory input to much of the emetic reflex arc neurocircuitry.[43] Serotonin, via 5-HT_3 receptors, and SP, via NK_1 receptors, increase the activity of vagal afferents.[44,45] Furthermore, other proemetic signals such as prostanoids have been found to increase excitability of vagal afferent neurons, thus potentiating their activity.[23,46,47] Although no prostaglandin receptors have been identified on confirmed emesis-related vagal afferents, immunolabeling for EP (prostaglandin E_2) prostaglandin receptors has been found in nodose ganglionic neurons.[48,49] On the other hand, CB_1 cannabinoid receptors and TRPV1 vanilloid receptors have been found on vagal afferent terminals and, as was the case for the ENS, these neurotransmitter systems reduce neuronal activity.[50,51] Vagal afferent terminals have been identified in both enteric nerve plexi. In the CNS, terminals were identified in the medial nucleus of the solitary tract (mNTS) and, to a lesser extent, in the rest of the dorsal vagal complex. In addition, several studies have observed branches of vagal afferents innervating the central pattern generator area, and possibly coinnervating the central pattern generator and dorsal vagal complex as well.[52,53]

2.4. Dorsal Vagal Complex

One of two key central mediators of the emetic reflex, the dorsal vagal complex is a cluster of nuclei in the dorsomedial medulla (see Figure 1). The area postrema (AP), which makes up the majority of the pharmacologically defined chemoreceptive trigger zone (CTZ), is a circumventricular organ that allows bloodborne chemicals (e.g., SP) absorbed by or secreted from the intestinal mucosa to bypass the blood−brain barrier and stimulate the dorsal vagal complex directly.[54,55] The AP/CTZ is populated by neurons containing a broad spectrum of neurotransmitter receptors, including dopaminergic, serotonergic, cholinergic, and cannabinergic receptors, resulting in sensitivity to a wide range of chemical signals. AP neurons are excitatory glutamatergic neurons and innervate the nucleus of the solitary tract (NTS).[56,57] The NTS, and specifically the medial subnucleus (mNTS; anatomically well-defined in larger animals), is the key integrative site for CNS modulation of the emetic reflex. It receives input from the AP and from vagal afferents, as well as diverse brain nuclei including the posterior and paraventricular hypothalamic nuclei and the serotonergic raphe nuclei. As with the AP, numerous receptor subtypes are present, including tachykininergic, serotonergic, dopaminergic, glutamatergic, and cannabinergic receptors.[7,49,58−68] Further enhancing its integrative abilities, primary neurons in the NTS have both glutamatergic and GABAergic phenotypes. After integrating the central and peripheral signals relating to emesis or other GI activity, both phenotypes of NTS neurons project to neurons in the DMNX and one or both types project to the central pattern generator. Activation of the NTS during emesis results in a biphasic response. In the initial phase, glutamatergic neurons excite DMNX motor output neurons, producing a retroperistaltic contraction in the intestine and a strong stomach contraction. However, the exact sequence of CNS and ENS neuronal activity required to produce the giant retrograde contraction that results in expulsion is still unclear. In the following phase, inhibitory NTS GABAergic projections and glutamatergic NTS projections that synapse onto DMNX inhibitory interneurons combine to suppress DMNX motor output, allowing relaxation of the gastric fundus and lower esophageal sphincter and opening of the physical pathway for expulsion of the toxin.[41,53,64,69−73] The final component of the dorsal vagal complex, the DMNX, consists of both motor neurons and interneurons. The motoneurons project to various parts of the gastrointestinal tract, including the stomach, lower esophageal sphincter, duodenum, and jejunum. As mentioned, both the motor and nonmotor phenotypes are involved in mediating neuronal output related to emesis. DMNX neurons have several serotonergic receptor subtypes, as well as dopamine $D_{2/3}$, tachykinin NK_1, and cannabinoid CB_1 receptors.[50,74,75] The DMNX also receives input from the central pattern generator in the ventrolateral medulla, a possible mechanism for coordinating emetic motor output with the prodromic effects of vomiting (e.g., salivation and increased sympathetic tone).[53,76]

2.5. Central Pattern Generator Area

Recent work has implicated a region of the ventrolateral medulla in the vicinity of the nucleus ambiguus (or retrofacial nucleus) in mediating the emetic reflex.[52,76,77] Electrophysiological studies have shown that central pattern generator neurons produce an intrinsic pattern of activity in relation to retching and emesis.[52] This pattern consists of a steady depolarization superimposed with rhythmic bursts of activity corresponding with retching motor contractions. Indeed, Fos immunoreactivity, a measure of neuronal activation, was found to increase in the central pattern generator in response to pseudoemetic stimuli induced by vagal afferent stimulation. Many of these neurons appear to be premotor neurons that, rather than innervating the gastrointestinal tract directly, innervate related areas such as the pharynx or unrelated areas that require coordination with the emetic reflex arc.[52,53,78−80] For example, respiratory activity must be coordinated with emesis to allow the chest musculature to contract during retching. Central pattern generator neurons project to ventral medullary respiratory neurons, which control phrenic nerve activity. In addition, central pattern generator neurons have been found to mediate the prodromal signs of emesis, in that during retching prior to emesis these central pattern generator neurons demonstrate a steady increase in firing rate until emesis actually occurs. This steady increase in firing results in a potent increase in salivation while retching occurs.

Connectivity of the central pattern generator has been studied primarily through electrophysiological recording. Although data are still limited, they indicate that collaterals from vagal afferents which innervate the dorsal vagal complex (especially the NTS) also innervate the central pattern generator. The central pattern generator appears to be heavily innervated with NK_1 receptor-containing fibers, and information from pharmacological studies suggests that glutamatergic neurotransmission via AMPA/kainate receptors is also involved.[53,61,77,81]

3. Dopamine: Synthesis, Storage, Release, Degradation, and Receptors

Dopamine (DA) is a neurotransmitter in both the CNS and the periphery. Some of the central functions of DA include control of locomotion, cognition, emotion, and neuroendocrine secretion.[82] In the periphery, DA is prominently involved in the kidney and vasculature to modulate sodium homeostasis and vascular tone. Significant evidence indicates that DA is also involved in the brainstem/gastrointestinal tract circuits associated with emesis. Similar to norepinephrine (NE) and epinephrine (EPI), DA is not only a catecholamine neurotransmitter in its own right, it is a precursor of these other neurotransmitters as well.[83] DA is mainly synthesized in the cytoplasm from the neutral amino acid tyrosine. A structurally based outline of DA synthesis and breakdown is presented in Figure 2. Briefly, tyrosine is initially converted to L-3,4-dihydroxyphenylalanine (L-DOPA) by the enzyme tyrosine hydroxylase. This oxidation reaction is the rate-limiting step in the production of not only DA but also all catecholamine neurotransmitters. L-DOPA is then converted to DA by aromatic amino acid decarboxylase. Although also known as L-DOPA decarboxylase, aromatic amino acid decarboxylase is not specific for this reaction and is expressed throughout the body in almost all cell types. In noradrenergic neurons, DA is converted by the enzyme dopamine β-hydroxylase. In turn, NE can be converted to EPI by phenylethanolamine *N*-methyltransferase. Once synthesized, DA in the cytoplasm is transported into secretory vesicles for storage and release. Upon nerve cell stimulation, the DA storage vesicles fuse with the plasma membrane to release DA into the synaptic cleft via exocytosis. In the synaptic cleft, DA can bind to different postsynaptic DAergic receptors and/or presynaptic dopaminergic D_2 autoreceptors. The D_2 autoreceptor reduces dopaminergic tone by inhibiting DA synthesis and release. Most released DA is transported back from the synaptic cleft into presynaptic neurons by a specific transporter, which can then either be cycled back into vesicles for reuse in or be degraded by monoamine oxidase enzymes (MAOs). MAOs exist in two isoforms: MAO-A is primarily found in the gastrointestinal tract, whereas MAO-B is more abundant in the brain.[84] However, either isoform, in conjunction with aldehyde dehydrogenase, can metabolize DA to dihydroxyphenylacetic acid. Extracellular DA that is not taken up into presynaptic cell can either diffuse out of the synaptic cleft or be degraded by catechol-*o*-methyltransferase. Catechol-*o*-methyltransferase is expressed in both the CNS and the peripheral tissues. The sequential action of catechol-*o*-methyltransferase and MAO enzymes converts DA to homovanillic acid. There are other quantitatively minor metabolites of DA, some of which may assume importance under certain conditions. At least six different forms of the DA receptors have been cloned from the brain.[82] The D_1-like class of DA receptors include D_1 and D_5 subtypes, wheras the D_2-like receptors are composed of D_{2S}-, D_{2L}-, D_3-, and D_4-subtypes. The D_1 class mediates an increase in the concentration of the cAMP second messenger, while the D_2-like class causes a reduction in cAMP levels.

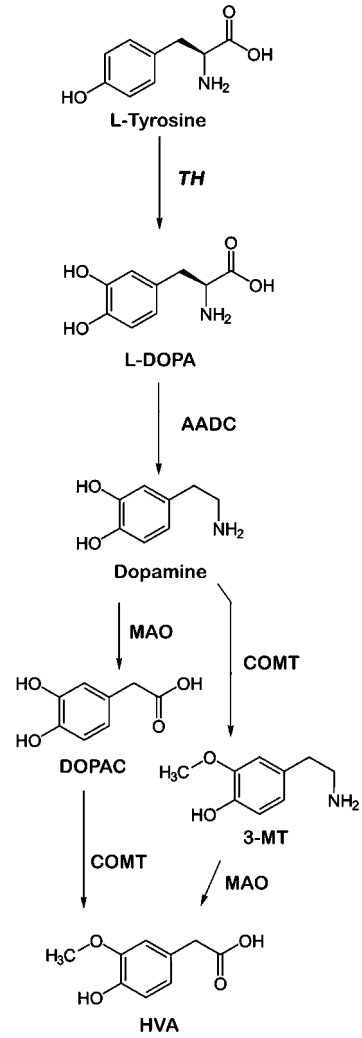

Figure 2. Biosynthesis of dopamine (DA). The rate-limiting enzyme for DA production (tyrosine hydroxylase) is italicized. Breakdown of DA is a two-step process, but the two sequential steps can occur in either order (via different intermediates) to obtain the primary breakdown product of DA, homovanillic acid. Note that DA can be acted upon by other enzymes to produce the other catecholamine neurotransmitters, norepinephrine and epinephrine (not depicted). Abbreviations: 3-MT, 3-methoxytyramine; AADC, aromatic L-amino acid decarboxylase; COMT, catechol-*o*-methyltransferase; DOPAC, dihydroxyphenylacetic acid; HVA, homovanillic acid; L-DOPA, L-3,4-dihydroxyphenylalanine; MAO, monoamine oxidase; TH, tyrosine hydroxylase.

3.1. Dorsal Vagal Complex

While D_1-like dopaminergic receptors (D_1 and D_5) are found in very low levels across the emetic loci in the dorsal vagal complex of mammalian medulla, D_2-like receptors (D_2, D_3, D_4), of which D_2 and D_3 subtypes are emetogenic, are heavily represented.[60] D_2 receptors are concentrated in the intermediate and medial subnuclei of the NTS and in the DMNX and AP, while D_3 receptors are more homogeneous across the entire NTS, DMNX, and AP. In contrast, D_4 receptors are found almost exclusively in the intermediate and medial subnuclei of the NTS and in the DMNX. While the vast majority of vagally associated catecholaminergic neurons in the DMNX are dopaminergic (i.e., exhibit tyrosine

hydroxylase but not dopamine β-hydroxylase immunoreactivity), only 10% of such neurons in NTS appear to be dopaminergic.[85] In addition, DA is present in specific nuclei of the dorsal vagal complex including AP, NTS, and DMNX.[86−89] Application of DA into the NTS inhibits neuronal activity in this nucleus.[90] Peripheral administration of dopamine $D_{2/3}$ receptor agonists causes emesis in the least shrew (*Cryptotis parva*), a well-characterized[91,92] small-animal model for emetic research that is necessary because traditional small-animal models (i.e., rats or mice) cannot vomit. Furthermore, these peripherally administered agonists induce Fos expression in the NTS and DMNX, but not in the AP, of this species.[93]

3.2. Vagal Afferents

Dopamine D_2 receptors and mRNA markers of DA synthetic enzymes are present in emesis related nodose ganglionic vagal afferents as well as on their central terminals in the NTS.[94,95] However, the majority of D_2 receptors in the NTS are located postsynaptically to the D_2 receptors on vagal afferent terminals. In addition, $D_{2/1}$-receptor stimulation by DA or selective D_2 agonists seems to indirectly increase the spontaneous activity of gastrointestinal tract vagal afferents via an increase in 5-HT turnover in the ileum.[95,96] On the other hand, subgroups of dopaminergic gastric projecting efferent DMNX neurons are either activated (via depolarization of D_1 receptors) or hyperpolarized (i.e., neuronal inhibition via stimulation of D_2 receptors), which indicates that DA plays important and complex roles in the control of gastrointestinal tract function.[85]

3.3. Enteric Nervous System

Because DA is the precursor of NE in the sympathetic innervation, it has been difficult to determine whether enteric DA is present in intrinsic neurons. Increasing evidence in these neurons (DA presence, dopaminergic D_1-, D_2-, D_3-, and D_5-receptors' mRNA and corresponding immunoblot proteins, tyrosine hydroxylase-, dopamine β-hydroxylase-, and enteric DA transporter mRNA, immunoreactivity, and colocalization) suggests that DA is an enteric neurotransmitter released from dopaminergic neurons in the mammalian gastrointestinal tract,[27,97] whose tyrosine hydroxylase activity can be regulated by intrinsic neuronal activity.[98] As within the dorsal vagal complex, intraperitoneal administration of selective $D_{2/3}$ agonists also induces Fos expression in the enteric neurons of the least shrew.[93] From behavioral and biochemical studies using genetically engineered knockouts, it appears that endogenous DA exerts a net inhibitory effect on intestinal motility and it does so primarily via neuronal D_2 receptors.[97]

3.4. Gastrointestinal Tissue

Despite significant evidence that DA modulates several important functions in the gastrointestinal tract (exocrine secretions, inhibition of intestinal motility, sodium absorption, and mucosal blood flow), the source and presence of DA in these functions have not been fully established.[99] However, the cited study shows that mesenteric organs (gastrointestinal tract, spleen, and pancreas) produce considerable amounts of DA with high turnover independent of the sympathoadrenal system. When combined with the cellular distribution of its rate-limiting enzyme, tyrosine hydroxylase, the overall published findings suggest the presence of a unique nonneuronal DA source in the mammalian gastrointestinal tract. The quantity of DA produced by the gastrointestinal tract is far too large to be simply derived from circulating L-DOPA. In humans and swine,[100] mesenteric organs may account for up to 46% of the DA formed in the body. Although the existing dogma suggests that tyrosine hydroxylase is located exclusively in catecholaminergic neurons in the brain, sympathetic nerves, and chromaffin tissue, more recent evidence from RT-PCR, in situ hybridization, and immunohistochemical techniques shows the presence of DA, DA transporter, and tyrosine hydroxylase in nonneuronal cells within the basal granulated cells of the mucosal epithelium along the entire extent of the small intestine of gerbils,[101] and in the denervated rat and human gastric mucosal and parietal cells.[102] Chemical sympathectomy reduces tissue concentrations of NE but does not have major effects on DA levels.[103] Moreover, D_1-D_5 receptor mRNAs and respective protein immunoblots have been found in the rat, mouse, and human gastric, duodenal, ileal, and colonic mucosa.[97,102,104] Interestingly, although D_4 receptors are restricted to the mucosa, ENS neurons failed to exhibit either the corresponding mRNA or immunoblot. DA evokes biphasic effects on the lower esophageal sphincter, with relaxation (a D_2-mediated effect) followed by a marked contraction via D_1 receptors in mammals.[105,106] DA also reduces gastric tone, intragastric pressure, antroduodenal coordination, and intestinal motility. The inhibitory effects are probably mediated via the inhibition of acetylcholine release via activation of D_2 receptors present on postganglionic cholinergic neurons as well as direct effects on gastrointestinal muscles.[105]

3.5. Dopamine and Emesis

The clinical use of dopamine D_2 antagonists in the gastrointestinal tract stems from (1) the ability of DA to induce emesis and lower esophageal sphincter relaxation (an event that occurs during vomiting), as well as DA's direct and indirect inhibitory effects on gastrointestinal motility (see above), (2) their efficacy as antiemetics in the prevention of nausea and vomiting during pregnancy, during migraine headaches, or following chemotherapy or surgery, and (3) their efficacy as prokinetics for the management of upper gastrointestinal tract motor disorders such as functional dyspepsia and gastric stasis of different origins.[105] Discovery of the role of DA in emesis began nearly six decades ago with a series of groundbreaking experiments by Wang and Borison.[107] They proposed that the site of emetic action of the direct-acting, nonselective DA agonist apomorphine, orally or intravenously administered, seemed to lie in the brainstem, as ablation of the AP/CTZ prevented the induced emesis in dogs, whereas vagotomy and abdominal sympathectomy failed to affect the response.[107] These findings were later confirmed as intracerebroventicular (i.c.v., 50 ug/animal) or intravenous (i.v., 0.1 mg/kg) injections of the nonselective D_2/5-HT_3 receptor antagonist metoclopramide in dogs were shown to prevent the vomiting produced by subcutaneously administered apomorphine, while vagotomy combined with splanchnectomy did not. In addition, the threshold emetic dose of i.c.v. apomorphine was 30−50 times lower than via the i.v. route.[108] Moreover, the CNS penetrable dopamine D_2 antagonist sulpride was a more effective antiemetic when administered i.v. versus i.c.v., but only when blocking emesis induced by i.v. apomorphine. When apomorphine was instead

administered i.c.v., sulpride was equally effective in blocking emesis regardless of its route of administration. Since DA is a polar molecule and cannot enter or leave the brain, this finding suggests that systemic release of endogenous DA activates peripheral emetic D_2 receptors in the AP on the blood side of the blood−brain barrier, whereas endogenous DA within the brain stimulates emetic D_2 receptors on the cerebrospinal fluid side of the blood−brain barrier. This notion was further substantiated by findings that (1) minor damage to the AP permanently abolished the emetic response to i.c.v. apomorphine but not to i.v. apomorphine;[108] (2) prevention of peripheral conversion of L-DOPA to DA by the peripheral decarboxylase inhibitor carbidopa concomitantly reduces blood DA concentration and emesis despite increasing brain DA levels, although vomiting is often not completely prevented in either patients,[109] dogs[110] or least shrews;[111] (3) the peripherally acting D_2 antagonist domperidone in doses up to 40 μg/kg can only partially reduce apomorphine-induced vomiting in beagle dogs, whereas the CNS permeable D_2 antagonist risperidone at 10 μg/kg completely prevented the vomiting;[112] and (4) peripheral injection of direct-acting and selective $D_{2/3}$ agonists causes emesis and induces Fos expression in the NTS and DMNX but not in the AP region of the least shrew (Ray, Chebolu, and Darmani, submitted for publication). This finding suggests that DA probably causes vomiting by acting on $D_{2/3}$ receptors located on neurons whose dendrites extend from the NTS into the AP. Because Fos is a nuclear antigen, only the cell bodies of the NTS neurons will express Fos and not their dendrites in the AP, while lesion of the AP could still lead to the prevention of DA-induced vomiting. An analogous hypothesis has been used to explain a similar pattern of Fos expression obtained in the ferret NTS and AP regions following loperamide-induced emesis.[113] Definitely, iontophoretic application of either DA or apomorphine within the AP causes neural excitation, indicating this region is probably responsive to emetic effects of dopaminergic agonists.[114] However, these DA agonists are nonselective and could cause neural excitation via nonemetic DA receptors. Certainly, apomorphine not only displays high affinity for dopaminergic D_2, D_3, and D_4 receptors and, to a lesser extent, for D_1 and D_5 receptors,[112] but also has emetic efficacy that can be route- and species-dependent.[115,116] Indeed, apomorphine's emetic potency varies across species with the following ED_{50} potency order: dog > man > ferret > cat = pigeon. Moreover, apomorphine does not induce vomiting in two monkey species (*Macaca mulatta* and *Macaca cynomolgus*), or the house musk shrew (*Suncus murinus*),[117] but it does in the least shrew.[118] Delineation of the roles of specific DA receptors has been hampered by the lack of highly selective agonists and antagonists as well as a comparative pharmacology of dopaminergic receptor subtypes. The emetic efficacy of a number of dopamine D_2 receptor agonists has now been firmly established in several vomiting species including ferrets,[119] least shrews,[118] and both *Cebus apella* and common marmoset (*Callithrix jacchus*) monkeys.[120,121] More recent studies also indicate a role for D_3 receptors in vomiting since a number of D_3-like agonists [e.g., 7-(OH)-DPAT] cause dose-dependent emesis in dogs, ferrets, and least shrews, which can be prevented by corresponding antagonists.[118,119,122] On the other hand, D_1/D_5 agonists such as SKF1297 or SKF38393 are not emetogenic in ferrets or dogs, and accordingly their antagonists (e.g., SCH 23390) fail to prevent apomorphine-induced emesis.[119,123,124] Likewise, agonists of D_4 receptors are devoid of emetic activity.[119] Thus, the preponderance of data suggests that DA (1) D_2 and/or D_3 receptor activation elicits emesis in diverse emetic species and (2) D_2-induced modulation of gastrointestinal motor activity such as relaxation of the lower esophageal sphincter and stomach (events that occur several minutes prior to the onset of the retrograde giant contraction in the small intestine) and inhibition of normal gastroduodenal coordination[105] probably contribute toward the expulsion of the vomitus. Thus, from the above discussion, it is apparent that dopamine $D_{2/3}$ antagonists possess antiemetic potential. Indeed, dopamine D_2 antagonists have been widely used for the prevention of nausea and vomiting during pregnancy, postoperative, during migraine attacks, and prior to chemotherapy exposure.[105] In addition, peripherally acting D_2 antagonists such as domperidone have been specifically used for the prevention of L-DOPA-induced emesis in patients with Parkinson's disease. The ability of these agents in counteracting gastric relaxation and intestinal motility may further add to their antiemetic potential.

4. Serotonin: Synthesis, Storage, Release, Degradation, and Receptors

5-Hydroxytryptamine [3-(β-aminoethyl)-5-hydroxyindole; 5-HT; serotonin] is an important signaling molecule in animals, man, and plants. Like DA, 5-HT is a monoamine neurotransmitter in both the CNS and the peripheral nervous system. Although the bulk of research aimed at understanding 5-HT function, release, uptake, and metabolism has focused on the CNS, over 95% of the 5-HT in the body is located in the periphery, especially in the gastrointestinal tract.[125,126] Of the peripheral 5-HT, ~90% is found in the gastrointestinal tract enterochromaffin cells (see Figure 1) and the remaining 10% is found in the gastrointestinal ENS. The rest of 5-HT (5%) is found in the CNS. Virtually all of the 5-HT in the blood is derived from the gastrointestinal tract. 5-HT is involved in the control and modulation of numerous physiological and psychological processes. In the CNS, 5-HT regulates mood, appetite, emesis, and migraine. In the gastrointestinal tract it generally plays a prokinetic role, and is an important mediator of sensation (e.g., nausea and emesis, satiety) between the intestine and the brain.[127]

The synthesis and breakdown of 5-HT is summarized in Figure 3. 5-HT is synthesized from the essential amino acid L-tryptophan in a two-step process: initial hydroxylation to 5-hydroxytryptophan (5-HTP) by tryptophan hydroxylase (TPH), then decarboxylation by the nonspecific enzyme aromatic amino acid decarboxylase to form 5-HT. TPH is the rate-limiting enzyme in 5-HT synthesis, and its distribution is limited to the cytoplasm of those tissues containing 5-HT. More recent findings show that TPH exists in two isoforms: TPH1, primarily expressed in enterochromaffin cells, and TPH2, expressed exclusively in neuronal cells such as the dorsal raphe and myenteric plexus.[128] Interestingly, the conversion of dietary L- tryptophan into 5-HT accounts for only 5% of its total metabolism, as L-tryptophan is largely converted in the liver to kynurenine by tryptophan pyrrolase. Within neurons, once 5-HT is synthesized in the cytoplasm, it is stored in vesicles in presynaptic terminals. Upon neuronal depolarization, 5-HT is released into the synaptic cleft via exocytosis. Once in the synaptic cleft, 5-HT can activate post- (e.g., 5-HT_3) and/or presynaptic (e.g., 5-HT_{1B}) serotonergic receptors. Reuptake is by means of a 5-HT specific transporter located in presynaptic nerve terminals

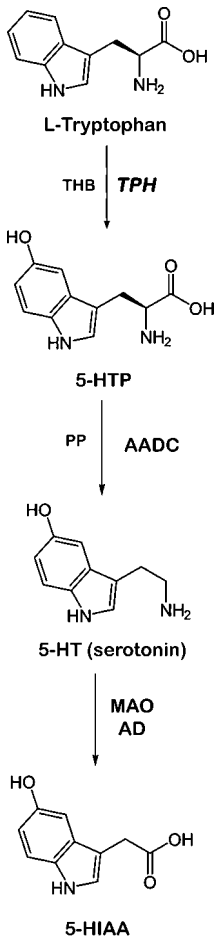

Figure 3. Biosynthesis of serotonin. The rate-limiting enzyme for serotonin production is italicized. The enzymes involved are to the right of the arrows, while necessary cofactors are to the left. The complete degradation of serotonin requires activity of both monoamine oxidase and aldehyde dehydrogenase. Serotonin can also be converted to melatonin in the pineal gland (not shown). Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytyramine (serotonin); 5-HTP, 5-hydroxytryptophan; AADC, aromatic L-amino acid decarboxylase; AD, aldehyde dehydrogenase; MAO, monoamine oxidase; PP, pyridoxal phosphate; THB, tetrahydrobiopterin; TPH, tryptophan hydroxylase.

(and also in nonneuronal cell membranes in both the CNS and the periphery) and serves as a major mechanism for the termination of action of synaptic 5-HT. After reuptake, 5-HT is recycled back into presynaptic storage vesicles, where it is protected from metabolism. Any free 5-HT is rapidly metabolized by cytosolic MAO to 5-hydroxyindole acetaldehyde, which is further metabolized either by aldehyde dehydrogenase mainly to 5-hydroxyindole acetic acid (5-HIAA) or by aldehyde reductase to a minor metabolite, hydroxytryptophol. 5-HT is metabolized by MAO-A in most tissues primarily, and to a smaller extent by MAO-B in platelets. The enterochromaffin cells of the gastrointestinal tract are specialized neuroendocrine cells that synthesize, store, and release 5-HT via a calcium dependent process. Enterochromaffin cells accumulate 5-HT in secretory vesicles via vesicular monoamine transporter-1. The enterochromaffin cell has a polarized structure with its apical membrane being covered by small microvilli, with 5-HT being located in secretory granules at its basolateral pole. 5-HT release from the basolateral surface can be triggered by mechanical and chemical stimulation or neuronal input in the gastrointestinal tract.[18] 5-HT is a major neurotransmitter and paracrine signal in the bidirectional communication between the CNS and the gastrointestinal tract. The released 5-HT from enterochromaffin cells in the intestinal mucosa is involved in motor, secretory, and sensory reflexes in the gut as well as the production of emesis. 5-HT produces its diverse effects via seven different families of serotonergic receptors (5-HT_1−5-HT_7), including multiple subtypes within the 5-HT_1 (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F}, and 5-HT_{1P}), 5-HT_2 (5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}), 5-HT_3 (5-HT_{3A}, 5-HT_{3B}, and 5-HT_{3C}), and 5-HT_5 (5-HT_{5A} and 5-HT_{5B}) families.[129] Of these, 5-HT_3 and 5-HT_4 and maybe 5-HT_{1A} are involved in emesis. All 5-HT receptors utilize G-proteins except 5-HT_3 receptors, which are part of the family of ligand-gated ion channels. The latter are permeable to Na^+, K^+, and Ca^{2+} ions and are formed by a pentameric complex. At least 5 different 5-HT_3 receptor subunits are known in humans.[130] Only 5-HT_{3A} subunits can form functional homo-oligomeric receptors, whereas 5-HT_{3B}, 5-HT_{3C}, 5-HT_{3D}, and 5-HT_{3E} cannot build a functional homopentameric receptor on their own. Although hetromeric 5-$HT_{3A/X}$ exhibits individual differences relative to homomeric 5-HT_{3A} receptors, 5-HT_3 receptor antagonists of diverse chemical structures and 5-HT_3 receptors of different compositions may exhibit significantly distinct pharmacological properties. Furthermore, the 5-HT_{3A}, 5-HT_{3B}, and 5-HT_{3C} subunits are almost ubiquitously expressed in the CNS and periphery. However, the 5-HT_{3D} is predominantly, and the 5-HT_{3E} subunit is exclusively, expressed in the gastrointestinal tract. These differences, in conjunction with the existence of peripheral (TPH1) and central (TPH2) isoforms of tryptophan hydroxylase (see above) and the susceptibility of some 5-HT_3 receptor antagonists to be more vigorously metabolized by patients who are rapid metabolizers,[131] provide clinical challenges that will have to be resolved in the coming years. Adding to the challenge, human primary 5-HT_4 receptor transcripts are spliced to produce up to 10 isoforms that differ in length and structure of their C-terminal tail,[132] making 5-HT_4 receptors more diversified. The functional specificity of these transcripts is not yet determined.

4.1. Dorsal Vagal Complex

Autoradiographic and homogenate radioligand binding studies have revealed a remarkably similar distribution of 5-HT_3 receptors, with significant densities across the dorsal vagal complex emetic nuclei of different mammalian species with the following density order: NTS ≫ AP > DMNX.[133] Both pre- and postsynaptic 5-HT_3 receptors are found in the dorsal vagal complex. Indeed, a variety of evidence suggest that 5-HT_3 receptors are localized presynaptically on nodose ganglion-sourced sensory vagal nerve terminals that coinnervate the gastrointestinal tract since vagotomy abolishes 5-HT_3 binding sites from the entire ferret brainstem.[134,135] Furthermore, electrical stimulation of abdominal vagal afferents or cisplatin exposure increases 5-HT tissue levels and turnover in the AP region of ferrets, whereas vagotomy or 5-HT_3 antagonist pretreatment attenuates cisplatin's effect on 5-HT release.[18,134] Involvement of 5-HT_3 receptors in the presynaptic modulation of other neurotransmitters such as glutamate is also well-recognized.[43] Other sources of 5-HT to the dorsal vagal complex region include the medullary raphe nuclei[136] and putative serotonergic cell bodies in the dorsal vagal complex such as the mNTS.[137] Not only are neurons within the AP region excited by 5-HT,[138] but postsynaptic serotonergic receptors (5-HT_{1A}, 5-HT_{2A}, 5-HT_3,

or 5-HT_4) in the dorsal vagal complex could also be activated following local 5-HT release.[139] Definitely, at the presynaptic level, 5-HT in the mNTS excites DMNX neurons by disfacilitation of inhibitory GABAergic neurons via activation of 5-HT_{1A} sites, whereas stimulation of postsynaptic DMNX 5-HT_{2A} receptors excites gastrointestinal tract-projecting efferent neurons to both the stomach and intestine.[140] Moreover, both 5-HT_3 and 5-HT_4 receptors are involved in the excitability of DMNX gastrointestinal tract-projecting neurons, generating long lasting facilitation of motility or prolonged gastric relaxation.[69] Although such electrophysiological studies indicate the presence of 5-HT_4 receptors in the brainstem, neither radioligand binding nor mRNA expression studies have evaluated expression of this receptor (or its homologues) in specific emetic loci in the dorsal vagal complex. Only limited published data exist on the role of selective 5-HT_3 agonists on Fos expression in the dorsal vagal complex. Recent evidence indicates that in the least shrew intraperitoneal injection of the 5-HT_3 receptor agonist 2-methyl-5-HT caused emesis as well as increased Fos expression not only in the well-accepted emetic nuclei of the dorsal vagal complex (AP, DMNX, and NTS) but also in the more recently recognized vomit locus, the central pattern generator, as well as in the dorsal raphe and paraventricular thalamic nuclei (Ray, Chebolu, and Darmani, submitted for publication).

Despite the extensive involvement of serotonergic neurotransmission in emesis, very little is known about the role of 5-HT in the central pattern generator area. 5-HT has been immunohistochemically identified in fibers in the central pattern generator area[141] (Darmani, unpublished data), and, although 5-HT_3 receptors are found in the central pattern generator area[142] and possibly 5-HT_{1A}, 5-HT_4, and 5-HT_7 receptors as well,[143] their function in relation to vomiting is unknown. However, as discussed earlier, peripheral administration of a 5-HT_3 selective agonist caused emesis as well as Fos expression in the central pattern generator area of the least shrew. In addition, these studies have described 5-HT activity in the vicinity of (or in) the nucleus ambiguus as a potent modulator of respiratory function. One could therefore postulate that increasing 5-HT activity in the central pattern generator would enhance expression of respiratory/prodromal signs of vomiting or perhaps tighten coupling between these signs and retching/vomiting behavior itself.

4.2. Vagal Afferents

Immunohistochemical and/or mRNA expression techniques indicate the presence of both 5-HT and 5-HT_3 receptors in vagal afferent fibers.[144−147] Electrical stimulation of vagal afferents,[148,149] intravenous injection of selective or nonselective 5-HT_3 receptor agonists, or administration of different emetics (cisplatin, $CuSO_4$, ouabain) can all induce dose-dependent increases in both abdominal afferent vagal nerve activity and the frequency of emesis in several vomiting species via either direct or indirect (subsequent to 5-HT release from enterochromaffin cells) stimulation of vagal afferent 5-HT_3 receptors.[18,91,150] The frequency of induced emesis parallels the change in vagal afferent activity, and both parameters are generally sensitive to 5-HT_3 receptor blockade and vagotomy. Ipecac syrup increases afferent vagal activity and induces vomiting via activation of vagal 5-HT_4 receptors.[151] Although electrophysiological evidence supports the presence of 5-HT_4 receptors on vagal afferents,[152] to date their presence has not been confirmed by either immunohistochemical or mRNA expression methods. However, administration of either a 5-HT_3 antagonist with 5-HT_4 agonist action (zacopride), the 5-HT_4 agonist 5-methoxytryptamine (5-MT), or $CuSO_4$ apparently causes emesis via activation of 5-HT_4 receptors.[153,154] Although the 5-MT-induced vomiting can be attenuated by vagotomy or by the 5-$HT_{3/4}$ antagonist ICS205-930, adminstration of 5-MT by itself did not potentiate vagal afferent activity,[155] nor did the 5-HT_4 antagonist, GR125487, prevent zacopride-induced emesis.[156] These differences need further experimental clarification to fully define a role for vagal 5-HT_4 receptor activation in chemotherapy-induced vomiting. In addition to its effects on vagal afferents, 5-HT participates in a vagal efferent pathway leading to the relaxation of the stomach.[125]

4.3. Enteric Nervous System

5-HT is synthesized, taken up in, and released by enteric nerves, particularly interneurons, which have signaling properties providing diverse motor, secretory, and sensory functions in the gastrointestinal tract. These interneurons innervate submucosal and myenteric nerves that respond to 5-HT via a variety of serotonergic receptors in several species. In guinea pigs, these interneurons constitute 2% of neurons in the myenteric plexus and their axons project anally to targets in other myenteric and submucosal ganglia.[157] Sensory and motor neurons both express a variety of 5-HT receptor subtypes, including 5-HT_1, 5-HT_3, and 5-HT_4.[158] 5-HT induces slow excitatory postsynaptic potentials (EPSPs) in enteric neurons as well as playing a minor role as a transmitter for fast EPSPs.[125] Acting via different receptors on somata of enteric motor neurons, 5-HT can make the bowel contract or relax. Indeed, via stimulation of 5-HT_3 and 5-HT_4 receptors on enteric cholinergic neurons, 5-HT releases acetylcholine to induce contraction of the smooth muscle, while activation of 5-HT_4, 5-HT_{1A}, or 5-HT_{1D} receptors on inhibitory enteric or nitrergic neurons causes release of nitric oxide to induce relaxation of intestinal smooth muscle. Thus, when intramural pressure increases, the enterochromaffin cells release 5-HT, which activates extrinsic vagal primary afferents and the intramucosal endings of submucosal or myenteric IPANs through 5-HT_{1P} receptors. Activation of IPANs initiates the peristaltic reflex pathways. The released 5-HT also activates presynaptic 5-HT_3 and 5-HT_4 receptors at IPAN terminals. This further facilitates propulsive activity in the guinea-pig colon, while only 5-HT_4 stimulation is involved in the human jejunal peristalsis.[159] Indeed, 5-HT_3 and 5-HT_4 receptors are found on presynaptic terminals of IPANs in several, but not all, species and synapse with a high proportion of myenteric neurons.[160] Activation of 5-HT_4 receptors enhances the release of both acetylcholine and calcitonin gene-related peptide (CGRP) from activated submucosal IPANs.[161] The increased presynaptic release of acetylcholine enhances fast neurotransmission, whereas enhancement of CGRP secretion potentiates the slow neurotransmission to follower cells in enteric ganglia. The latter effect is required for the spread of excitation within the submucosal plexus, and these effects enhance peristaltic propulsion and secretion. Intraperitoneal injection of the selective 5-HT_3 agonist 2-methyl-5-HT induced Fos expression in the enteric nerves as well as vomiting in the least shrew, which suggests that changes in intestinal motility may also contribute to the production of emesis (Ray, Chebolu, and Darmani, submitted for publication). The substantial connections from serotonergic descend-

ing interneurons to both myenteric and submucosal cholinergic secremotor neurons suggests that these interneurons are important for the regulation of intestinal secretion.[157] However, while 5-HT_3 receptors are abundant, 5-HT_4 receptors are almost entirely absent from secretomotor cholinergic neurons.[157,160]

4.4. Gastrointestinal Tissue

Over 90% of peripheral 5-HT is produced by enterochromaffin cells in the gastrointestinal tract epithelium. In some species, mast cells also contain high levels of 5-HT. 5-HT is highly charged at physiological pH and thus does not cross plasma membranes in the absence of a transporter. Unlike acetylcholine, which can be inactivated by extracellular acetylcholine esterase, no extracellular metabolic enzyme exists for 5-HT. Thus, termination of action of 5-HT requires its catabolism, which is dependent upon the presence of myenteric and intestinal enterocytic serotonin transporter. 5-HT not only transduces information to intrinsic primary afferent neurons but also to adjacent cells in the mucosa and submucosa.[162] Control of gastrointestinal motility requires the coordinated activity of different cell types such as nerves, smooth muscle cells, and ICC, which are specialized mesenchymal cells essential for normal gut motility. ICC express several different serotonergic receptors including 5-HT_{2B}, 5-HT_3, and 5-HT_4 subtypes in rodents. They generate spontaneous, rhythmical electrical oscillations, the slow waves, which are the pacemakers of the gastrointestinal tract. The ICC are involved in the conduction and amplification of the above-described neuronal signals from excitatory cholinergic and inhibitory nitrergic motor neurons. Serotonergic 5-HT_{2B}, 5-HT_4, and 5-HT_7 receptors are expressed on enteric smooth muscle cells. Activation of 5-HT_7 receptors causes relaxation, while stimulation of 5-HT_{2B} receptors increases muscle activity. 5-HT_4 receptor excitation appears to mediate both inhibition and excitation of smooth muscle cell activity either directly or indirectly via neurons. 5-HT does not play a significant role in pathways to longitudinal motor neurons that receive input (nitrergic interneurons) from descending reflex pathways expressing 5-HT_4 receptors.[157] On the other hand, excitatory circular motor neurons receive substantial input from serotonergic interneurons, since descending excitation is substantially depressed by 5-HT_3 receptor antagonists in guinea pig ileum. Furthermore, while 5-HT_4 receptors are present on circular muscle, longitudinal muscles lack these receptors.[160] 5-HT_4 receptors help to regulate normal gut motility, and its agonists are used clinically as prokinetic agents.[163]

4.5. Serotonin and Emesis

Peripherally administered 5-HT is a potent and efficacious emetogen in the least shrew[91] and the house musk shrew,[164] but not in the often used ferret model of emesis.[165] On the basis of the presumption that 5-HT is highly charged at physiological pH and thus should not cross the blood−brain barrier, this emetogen is generally thought to initiate vomiting in the periphery via the well-accepted and 5-HT-based theory of acute chemotherapy-induced vomiting (see section 9.1.1), in which 5-HT initially stimulates 5-HT_3 receptors on vagal afferents in the gastrointestinal tract, which then activate the dorsal vagal complex emetic circuits to cause vomiting.[150] Indeed, not only has vagotomy been shown to prevent 5-HT-induced emesis in house musk shrews, but peripheral

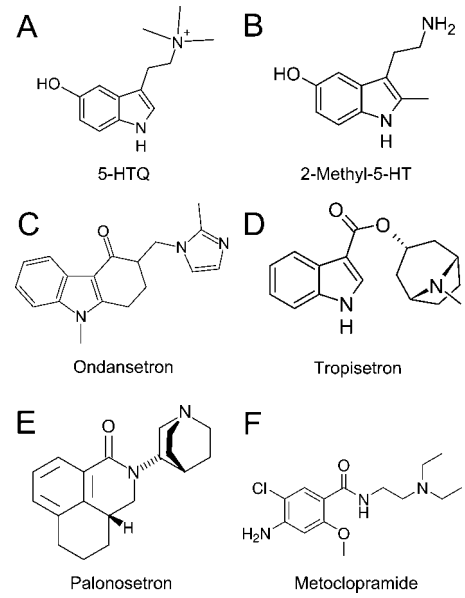

Figure 4. Structural relationships of emesis-modulating serotonergic compounds. (A) The quaternary ionic form of 5-HT, *N*,*N*,*N*-trimethylserotonin (5-HTQ) is a nonbrain-penetrant, 5-HT_3 receptor agonist. (B) 2-methylserotonin is a brain-penetrant, 5-HT_3 receptor agonist. Both 5-HTQ and 2-Me-5-HT are potent emetogens. The compounds in (C)−(F) are all antiemetics. (C) Ondansetron is the prototypical "setron" 5-HT_3 receptor antagonist and representative of the first-generation carbazole-derived antagonists. (D) Tropisetron is an indole-derived, first-generation antagonist with similar affinity and a longer half-life (over 40% longer) than ondansetron. (E) Palonosetron is a second-generation, isoquinoline-derived 5-HT_3 antagonist with an ~40 h half-life (about 10-fold greater than ondansetron). It is the only 5-HT_3 antagonist that has some efficacy against (and is approved for use to treat) the delayed phase of chemotherapy-induced vomiting. (F) Metoclopramide, an early antiemetic not related to the setrons, was found to have 5-HT_3 antagonist activity in high doses. It also has significant D_2 dopamine receptor affinity, as described in Figure 9. Abbreviations: 2-Me-5-HT, 2-methyl-5-hydroxytryptamine, 2-methylserotonin; 5-HTQ, *N*,*N*,*N*-trimethylserotonin, 5-HT quaternary form.

injections of both brain-penetrating (such as 2-methyl-5-HT and mCPBG) and nonpenetrating (e.g., 5-HTQ) selective 5-HT_3 receptor agonists (described in Figure 4A/B) induce vomiting in a 5-HT_3 antagonist-sensitive manner in both shrew species. Along with such evidence, both 5-HT and 2-methyl-5-HT, as well as indirect-acting serotonergic drugs (e.g., cisplatin), increase abdominal vagal afferent activity in the ferret.[38] Serotonin may also indirectly contribute to emesis via the discussed alterations in gastrointestinal tract motility, since 2-methyl-5-HT has been shown to increase Fos expressions in the ENS of the least shrew (Ray, Chebolu, and Darmani, submitted for publication). Since intraperitoneal 2-methyl-5-HT (and also cisplatin) has been shown to cause strong Fos expression in the AP of least shrews (Ray, Chebolu, and Darmani, submitted), it is possible that 5-HT could activate the blood side of the AP in the brainstem to induce vomiting. However, this process does not appear to be a viable mechanism since: (1) lesion of the AP in cats failed to affect the emesis induced by either the systemically administered 5-HT_3 receptor agonist, phenylbiguanide, or by cisplatin,[166] and (2) direct injection of 2-methyl-5-HT into the area postrema of ferrets failed to induce a complete emetic response.[167] On the other hand, equally important but often ignored diverse lines of evidence indicate that, in addition to the well-accepted peripheral pathway of emesis, central serotonergic mechanism(s) may exist. For example:

(1) Quaternary analogues of brain-permeable drugs generally do not permeate the blood−brain barrier efficiently. The quaternary form of 5-HT, 5-HTQ, is a nonpenetrable selective 5-HT_3 receptor agonist that has 10 times greater affinity than 5-HT for 5-HT_3 sites. Following intraperitoneal injection, 5-HTQ was unexpectedly found to be three times less potent than 5-HT in causing emesis in the least shrew, whereas the brain-penetrable selective agonist, 2-methyl-5-HT, was a more potent emetogen than 5-HT in both shrew species.[91,164,168] (2) Selective systemic inhibition of 5-HT synthesis in the periphery by inhibitors of TPH1 caused a modest decrease in cisplatin induced vomiting in ferrets.[128] (3) Vagotomy in cats enhances the emetic efficacy of both the direct-acting 5-HT_3 receptor agonist phenylbiguanide and the indirect agonist cisplatin.[166] (4) Blockade of conversion of systemic 5-HTP to 5-HT by the peripheral decarboxylase inhibitor, carbidopa, attenuates the frequency of 5-HTP-induced emesis in the least shrew (i.e., a peripherally mediated 5-HT_3-receptor event) by only 50%, and further reduction in vomiting frequency (due to activation of central 5-HT_3 receptors) requires administration of larger centrally active doses of carbidopa.[168] (5) The phytocannabinoid Δ^9-tetrahydrocannabinol (Δ^9-THC) reduces the frequency of 5-HTP-induced emesis in a biphasic manner, and the inhibition is significantly more potent against its central component.[168] (6) i.c.v. administration of 5-HT or ciplatin at peripherally ineffective doses causes emesis in marmoset monkeys, cats, and pigeons.[120,169,170] (7) Direct microinjection of 5-HT_3 receptor antagonists in discrete dorsal vagal complex emetic loci in the brainstem prevents cisplatin induced emesis in ferrets, dogs, and cats.[167,169,171] (8) Intraperitoneal administration of 2-methyl-5-HT induces Fos expression in brainstem emetic nuclei including the AP, NTS, DMNX, and central pattern generator (Ray, Chebolu, and Darmani, submitted for publication). 5-HT_3 receptor antagonists have a narrow spectrum of antiemetic activity in different animal models of emesis, being most successful against the immediate phase of chemotherapy-induced vomiting[150] and anesthesia-related vomiting.[172] Indeed, they lack efficacy against vomiting caused by (1) lipopolysaccharide in piglets,[173] (2) phosphodiesterase IV inhibitors in ferrets,[174] (3) cardiac glycosides in house musk shrews,[175] (4) ethanol in house musk shrews,[176] (5) copper sulfate-induced vomiting in ferrets or house musk shrews,[177,178] and (6) motion sickness in house musk shrews.[179] The clinical efficacy of these agents is also confined to the immediate phase of chemotherapy-induced vomiting[9] and postoperative nausea and vomiting.[180]

The role of 5-HT_4 receptor activation in emesis as determined by agonist dose−response studies has not yet been fully explored, and most data implicating its emetogenicity are derived from indirect 5-$HT_{3/4}$ antagonist studies involving either $CuSO_4$-, zacopride-, or chemotherapy-induced emesis.[153,154,179,181] Although 5-HT_4 receptor antagonists are not effective against acute cisplatin induced emesis when administered alone, they significantly potentiate the antiemetic effects of 5-HT_3 antagonists in acute chemotherapy-induced vomiting in both least and musk shrews[182] (Darmani, unpublished data). Stimulation of 5-$HT_{3/4}$ receptors in the dorsal vagal complex causes excitation of gastrointestinal tract-projecting neurons in the NTS and DMNX.[140,183] 5-HT_4 receptor activation negatively modulates an A-type potassium current, which enhances excitability in DMNX neurons.[69] This would enhance the ability of 5-HT to induce firing of DMNX neurons via 5-HT_3 receptor activation,[69,140] but not necessarily increase firing directly. This mechanism can explain the lack of effect of the 5-HT_4 antagonist SDZ-205557 on emesis when administered alone, as well as its ability to potentiate the antiemetic activity of 5-HT_3 antagonists. A "selective" 5-HT_4 agonist, 5-MT, has been shown to induce vomiting following large oral doses but not after a low intravenous dose.[153] The emetic effect is suggested to be due to stimulation of 5-HT_4 receptors on vagal afferents since the induced vomiting was inhibited by bilateral vagotomy and, second, because 5-HT-induced depolarization in the rat isolated abdominal vagus nerves can be antagonized by the selective 5-HT_4 antagonist, SB204070.[184] 5-HT_4 receptors in the periphery help to regulate normal gut motility, and its agonists are used clinically as prokinetic agents.[163] The latter effect may also contribute to prevention of vomiting. Thus, changes in the activity of both central and peripheral 5-HT_4 receptors could modulate emesis.

Unlike the proemetic 5-$HT_{3/4}$ receptors, stimulation of 5-HT_{1A} receptors by different full and partial agonists seems to exert antiemetic activity in diverse species against several emetic stimuli including: motion, nicotine, cisplatin, $CuSO_4$, veratrine, cisplatin, mechanical stimulation of the upper gastrointestinal tract in the house musk shrew;[185,186] motion, cisplatin, or xylazine in the cat;[187−189] ipecac, emetine, mCPBG, cisplatin, or cyclophosphamide in pigeons;[190,191] and cisplatin.[192] However, in the clinical setting, 5-HT_{1A} agonists are found not to be an effective antiemetic in cancer patients receiving chemotherapy.[193] They also cause side effects such as nausea and vomiting in patients when taken as medication for conditions such as depression.[194−197] Species differences and dopaminergic agonism of some compounds have already been suggested as possible explanations for the opposing basic and clinical findings.[198] However, another possible explanation is that at the antiemetic doses used in animals, 5-HT_{1A} agonists most likely induce serotonin syndrome. Serotonin syndrome is most prominent in rats in response to administration of 5-HT_{1A} agonists such as 8-(OH) DPAT[199] but can occur in diverse species including the least[200] and house musk shrews,[186] dogs,[201] cats,[202] and humans.[203] The main features of serotonin syndrome in the rat include persistent behaviors such as: hindleg abduction, lateral head-weaving, forepaw treading, flat body posture, rollover, tremor, straub tail, and hypothermia. Most of these behaviors can occur in other species upon activation of 5-HT_{1A} receptors. Thus, behavioral competition with serotonin syndrome could antagonize the emetic process since some components of serotonin syndrome behaviors are very intense at the antiemetic doses used in the cited animal studies.

Although some inter- and intraspecies differences occur in both the distribution of 5$HT_{3/4}$ receptors (both in the CNS and peripheral emetic loci) and in emetic and antiemetic potential of individual 5-$HT_{3/4}$ agonists and antagonists, significant direct and indirect evidence exists to support the notion that vagal afferent activation is not an exclusive avenue to 5-HT induced emesis. The diverse effects may arise from differences in predominant pathways that induce emesis. For example, a given emetogen may induce vomiting by parallel mechanisms, whereby one pathway may be predominant for a specific stimulus or species, or may become predominant during the time course of chemotherapy-induced vomiting or as a result of manipulations such as vagotomy.[166,204] The above discussion is consistent with the notion that both directly and indirectly (e.g., 5-HT precursors,

releasers, and uptake inhibitors) acting serotonergic 5-$HT_{3/4}$ agonists induce emesis in both animals and man.[166]

5. Substance P: Synthesis, Storage, Release, Degradation, and Receptors

The tachykinin peptide superfamily represents one of the largest peptide families in the animal kingdom. Thus far, more than 40 tachykinins have been discovered from invertebrates (e.g., insects, worms), prevertebrates (e.g., *Amphioxus lanceolatus*), submammalian vertebrates (e.g., amphibians, reptiles), and mammalian tissues.[205−207] The mammalian tachykinins include SP, neurokinin A (NKA), and neurokinin B (NKB). In addition, the N-terminally extended forms of NKA, neuropeptide K, and neurokinin γ are also biologically active. Although tachykinins were considered almost exclusively as peptides of neuronal origin, they are also found in endothelial cells, Leydig cells, inflammatory and immune cells, enterochromaffin cells, smooth muscle cells, and placental tissue. Mammalian tachykinins are derived from two tachykinin precursor genes: four of these peptides (SP, NKA, neuropeptide K, and neurokinin γ) are encoded by the tachykinin precursor 1 (TAC1) gene [originally called preprotachykinin-A (PPT-A)], while the TAC3 gene (originally termed PPT-B) encodes the sequence of NKB. Synthesis of SP and other TAC1-generated peptides is outlined briefly in Figure 5. The human TAC1 gene consists of seven exons, and the sequences that encode SP and NKA are contained in exons 3 and 6, respectively. The sequence encoding neurokinin γ is contained in exons 3, 5, and 6, and the encoding sequence for neuropeptide K is contained in exons 3, 4, 5, and 6. The transcription of the TAC1 gene generates a pre-mRNA that is spliced into four different mRNA isoforms (α, β, γ, δ) that differ in their exon combinations. The β form of PPT-A mRNA contains all seven exons of the corresponding gene, while the α-PPT-A mRNA lacks exon 6, γ-PPT-A lacks exon 4, and δ-PTT-A lacks exons 4 and 6. Thus, the SP precursor sequence is synthesized from all four isoforms, whereas the NKA sequence is present in β and γ PPT-A mRNAs, the neurokinin γ sequence is present in γ-PPT-A mRNA, and the neuropeptide K sequence is only coded by the β-PPT-A isoform. Subsequent translation of these mRNAs as well as their post-translational processing gives rise to the cited tachykinins.

Tachykinins can activate three specific membrane receptors known as NK_1, NK_2, and NK_3, which belong to the family of G protein-coupled receptors and have been cloned in several species including humans. The human NK_1 and NK_2 receptors are proteins containing 407 and 398 amino acids, respectively, while the NK_3 receptor is longer with 465 residues. These receptors are recognized with moderate selectivity by endogenous SP, NKA, and NKB, which act as full agonists with preferential selectivity for NK_1, NK_2, and NK_3 receptors, respectively. However, the differences in their affinities are not large, and thus cross-interaction with different tachykinin receptors leading to some agonist promiscuity is a real possibilty.[208] Moreover, there are marked species-dependent differences in the pattern of expression of the tachykinin receptor types in a given tissue, suggesting that different receptors may exert similar functions.[205] Tachykinin receptors couple with: (1) Gq-protein and binding with ligands results in production of 1,4,5-inositol triphosphate and elevation of intracellular Ca^{2+} through activation of phospholipase C and (2) Gs-protein that leads to production of cAMP. Activation of NK_1 receptors leads to emesis,[92] and stimulation of NK_{1-3} receptors affects gastrointestinal motility and secretion.[28,36,207,208]

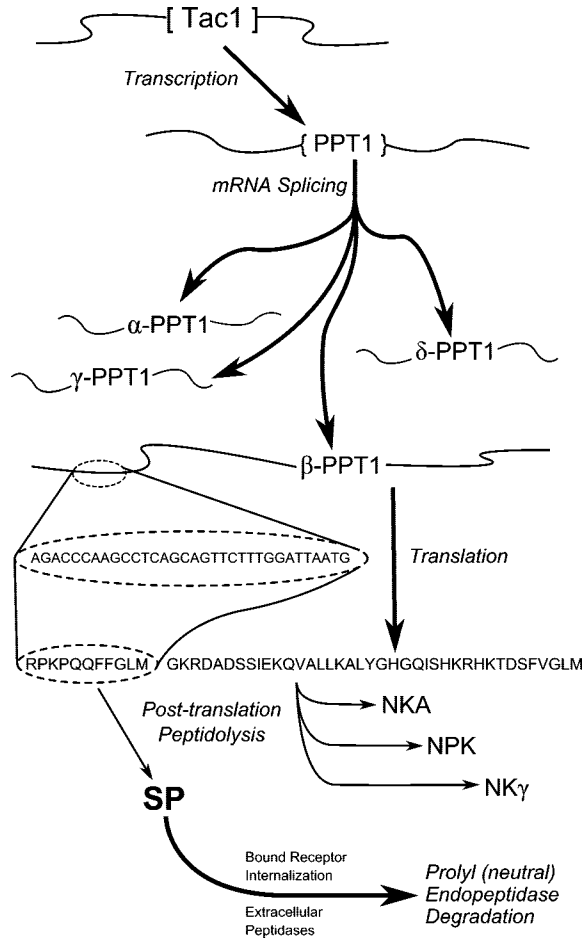

Figure 5. Biosynthesis of substance P (SP). SP is initially transcribed into a single prepro-mRNA, preprotachykinin-1, which is spliced into multiple isoforms. All isoforms can produce SP, but for clarity, only the further processing of the β isoform is depicted. The sequence of least shrew β-PPT1 that is translated into SP is enclosed in the upper dashed oval, and its translated peptide sequence is enclosed in the lower oval. Post-translational modification (hydrolysis) of β-PPT1 generates SP and several other neuroactive peptides. Excess extracellular (i.e., synaptic) SP, or receptor-bound SP that has been internalized, is degraded by endopeptidases, and the amino acid residues are recycled. Abbreviations: NKA, neurokinin A; NKγ, neurokinin gamma; NPK, neuropeptide K; PPT1, preprotachykinin-1; SP, substance P.

5.1. Dorsal Vagal Complex

Since the NTS is the primary relay of visceral sensory afferents (e.g., cardiovascular, respiratory, skeletal muscle, and gastrointestinal systems) in the CNS, considerable integration of autonomic and central input as well as interneuronal communication between different cell populations occurs in this nucleus of the dorsal vagal complex.[209] Tachykinins are considered important neurotransmitters in the dorsal vagal complex and high levels of SP and SP-like immunoreactivity are found in the AP, NTS, and DMNX of several emetic and nonemetic species.[208,210,211] Likewise, significant tissue concentrations of NKA and lower tissue concentrations of NKB as well as their corresponding neurokinin-like immunoreactivity are found in the brainstem.[212,213] Depending upon the species, SP-containing immunoreactive fibers are distributed to the ventral and/or lateral borders of

the AP, whose cell bodies are thought to be in the NTS and/or nodose ganglion (vagal afferents). However, SP-containing cell bodies are not present in the AP. The NTS has reciprocal neuronal links with the AP as well as other brainstem nuclei. SP-like immunoreactivity is found in both nerve fibers and terminals in the NTS, and the most prominent staining occurs in the medial, the commissural, and the subnucleus gelatinosus regions of the NTS (see also figure 1). The immunoreactivity in the medial and subnucleus gelatinosus regions is of particular interest in emesis since these are sites of termination of gastrointestinal vagal afferents that bring information from the gastrointestinal tract to the brainstem. Vagal efferents arising from the DMNX innervate the entire gastrointestinal tract and SP via NK_1 receptors, which play an important neurotransmitter role in inhibiting gastric motility.[72] The presence of SP-like immunoreactivity in nerve fibers in the DMNX is suggestive of afferent projections to this structure, while immunoreactive cell bodies indicate that SP is a neurotransmitter in some preganglionic vagal efferent fibers.[210] NK_1 receptors are well-expressed in the dorsal vagal complex emetic nuclei with high expression levels in the subnucleus gelatinosus of the NTS and dorsal NTS, as well as in the DMNX, and substantially lower levels in the AP region of the ferret and rat brain.[210] NK_1 receptors are involved in the excitation of NTS and DMNX neurons.[214] Furthermore, intraperitoneal injection of the brain-penetrating NK_1 agonist GR73632 causes emesis as well as Fos expression in the NTS and DMNX but not in the AP of the least shrew brainstem.[92] Moderate NK_3 immunoreactivity has been observed in the rat NTS and DMNX.[208] The central pattern generator area, adjacent to the nucleus ambiguus, is heavily innervated by SP-containing afferents in many species, including the rat,[215] dog,[81] ferret,[53,216] human,[212] and least shrew.[217] Much of this data arose from basic anatomical research and was not directly correlated with vomiting. However, several studies exist that correlated emetic behavior with the central pattern generator and neighboring areas, as well as with SP neurotransmission. Electrophysiological data demonstrated that central pattern generator neurons driven by the mNTS,[62] as well as nearby neurons associated with the prodromic signs of vomiting (i.e., hypersalivation, tachypnea), fired in a rhythmic pattern synchronized to the retching and vomiting motor sequences induced by vagal stimulation.[53,78,218] In the decerebrate dog model, this neuronal activity was abolished along with the actual emesis-associated behaviors upon application of NK_1 receptor antagonists,[70,81] a finding recently reproduced in the ferret.[53] In addition, intraperitoneal administration of GR73632 increases Fos expression in the central pattern generator of vomiting least shrews, which suggests that this locus is an important anatomical emetic substrate of SP (Ray, Chebolu, and Darmani, submitted for publication).

5.2. Vagal Afferents

The cell bodies of the sensory nodose ganglion express mRNA for the SP precursor, preprotachykinin, and synthesize SP, which is then bidirectionally transported via vagal axons to the brainstem as well as the gastrointestinal tract, resulting in its release.[219−221] SP and NKA, but not NKB, immunoreactivity in these afferents has been demonstrated in several emetic and nonemetic species including humans.[147,210] The released SP in the gastrointestinal tract plays an important peripheral role in gastrointestinal motility reflexes, which could indirectly modulate emesis.[28,36,92] In addition, SP stimulation of vagal afferents occurs in an NK_1 antagonist-sensitive manner, and thus may directly induce vomiting through subsequent activation of emetic circuits in the dorsal vagal complex.[38,92] Although electrophysiological studies indicate the presence of NK_1 receptors on the gastrointestinal tract vagal afferents in several species including ferrets,[38,44] currently no mRNA expression or immunohistochemical evidence exists for their presence.

5.3. Enteric Nervous System

The mammalian gut contains both SP and NKA but appears to lack the gene to produce NKB.[28,36,207,208] Intrinsic enteric neurons whose cell bodies reside within the myenteric and submucosal ganglia have a dense supply of tachykinin containing fibers that not only connect the two plexi with each other but also supply the longitudinal muscle, circular muscle, and muscularis mucosae in the intestinal wall. Although there are regional and species differences in the density of tachykininergic innervations of the gastrointestinal tract, the general distribution of SP/NKA containing somata and axons are very similar. There appear to be at least eight types of tacyhkinin-containing neurons, six of which are intrinsic. Intrinsic interneurons located in the submucosal ganglia supply the villi and have collaterals to myenteric ganglia. Five projections arise from the myenteric plexus, a very short projection ending within the same row of ganglia or within adjacent rows of ganglia on both sides, a longer projection within the myenteric plexus, a projection to the circular muscle, a projection to the submucosal ganglia where the axons surround most of the submucosal nerve cell bodies, and a projection to the villi. Myenteric IPANs have tachykinin immunoreactivity and project locally, to provide dense networks of terminals in ganglia close to their cell bodies, as well as projecting to the submucosa and mucosa. Extrinsic primary afferent neurons are another source of tachykinins that project to submucosal blood vessels and to enteric ganglia. Tachykinins are involved not only in local reflexes but also in the transmission of sensory information from the gastrointestinal tract to the brainstem.

NK_1 receptors are present in enteric neurons of diverse species[207] including: (1) nitric oxide synthase-immunoreactive inhibitory motor neurons, (2) choline acetyltransferase/tachykinin-immunoreactive excitatory neurons to the circular muscle, (3) choline acetyltransferase/neuropeptide Y/somatostatin-immunoreactive secretomotor neurons, (4) choline acetyltransferase/calbindin myenteric IPANs, and (5) choline acetyltransferase/tachykinin submucosal IPANs. The locations of these are congruent with a role of NK_1 receptors in regulating motility, neuronal excitability, and mucosal water and ion transport. This conclusion is further supported by the recent finding that the selective NK_1 agonist GR73632 increases Fos expression in the enteric neurons of the least shrew (Ray, Chebolu, and Darmani, submitted for publication). In the myenteric ganglia, NK_3 receptors are found on the (1) IPANs, (2) excitatory motor neurons and ascending motor neurons, (3) inhibitory motor neurons, (4) descending interneurons, and (5) secretomotor neurons. In the submucosal ganglia, NK_3 receptors are present on (1) secremotor/vasodilator neurons, (2) secremotor neurons, and (3) cell bodies in myenteric and submucous plexi of the small and large intestines.[207,222] In terms of function, both NK_1 and NK_3 receptors are involved in neuro−neuronal transmission in the enteric nervous system. Tachykinins have roles in the

generation of slow EPSPs that are mediated by NK_1 and NK_3 receptors in the IPANs, and in both ascending and descending pathways affecting motility.[207,222] Indeed, transmission at the first synapse between the IPAN and interneurons following the stimulation of mucosal reflexes within descending motility-controlling pathways involves tachykinins via the activation of NK_3 receptors, whereas transmission from IPANs to the inhibitory muscle motor neurons involves NK_1 receptors. In addition, IPANs make synaptic connections with other IPANs via tachykinins through NK_1 and NK_3 receptors.

5.4. Gastrointestinal Tissue

The tissue concentrations of tachykinins are generally low in the esophagus, intermediate in the stomach, and high in the intestine in most species.[28,36] Species differences also occur with regard to the concentrations of SP and NKA in different layers of the gastrointestinal wall, with the external muscle layer having higher concentrations than the mucosal/submucosal layer, although similar levels are seen in the human and equine intestines. Apart from the discussed intrinsic and extrinsic neuronal sources of tachykinins in the gastrointestinal tract, three other tachykinin sources include endocrine cells within the gastrointestinal tract epithelium, endothelial cells, and blood-derived or resident immune cells in the lamina propria of the gastrointestinal tract mucosa. The endocrine cells are a population of 5-HT-containing enterochromaffin cells that occur throughout the gastrointestinal tract. Some epithelial cells separate from the enterochromaffin cells also express and release SP in the human colon.

Tachykinins enhance motor activity in all regions and layers of the gastrointestinal tract.[28,36,207] Often this action depends not only on a direct activation of the muscle but also on stimulation of enteric motor neurons that excite the muscle via release of acetylcholine. Indeed, acetylcholine is the primary transmitter of excitatory neurons innervating the muscle, while SP and NKA are cotransmitters of these excitatory neurons. Both NK_1 and NK_2 receptors mediate the transmission from excitatory motor neurons to muscle. Excitation of muscle involves indirect activation through NK_1-receptor-expressing ICC and direct effects on smooth muscle via NK_2 receptor stimulation. Besides their prominent excitatory action, tachykinins can also exert NK_1 receptor-mediated inhibitory influences either through IPAN transmission on inhibitory muscle motor neurons or through interrupting excitatory relays. A wide variety of pharmacological, immunohistochemical, and mRNA expression studies indicate that the mucosal epithelial cells of the gastrointestinal tract express NK_1 and NK_2 receptors. Both IPANs and spinal primary afferent neurons release tachykinins from their nerve endings on mucosal NK_1 and NK_2 receptors, leading to increased fluid secretion. This can contribute to inflammatory reactions by acting on lymphocytes and other immune-related cells. Indeed, NK_1 and NK_2 receptor immunoreactivity is present, respectively, on lymphocytes and some inflammatory cells of the lamina propria, which are involved in the regulation of gut immune and inflammatory responses. Since tachykinin receptor antagonists do not produce major gastrointestinal side effects, it has been suggested that neither NK_1 nor NK_3 receptors seem to play major roles in normal GI physiology but can be activated in an activity-dependent manner, indicating roles in defensive and/or pathological GI functions.[208] This conclusion should be taken with caution, since selective ablation of NK_1 receptors from a small portion of the least shrew small intestine with the targeted toxin stable substance P-saporin (SSP-SAP) not only reduced NK_1 receptor-mediated emesis but was fatal 5 days post toxin treatment.[92]

5.5. Substance P and Emesis

Important impetuses behind the development of SP-based antiemetics have been the failure of 5-HT_3 receptor antagonists to prevent the delayed phase of chemotherapy-induced vomiting, as well as a need for more broad-spectrum antiemetics in the clinic. Initial evidence in support of an emetic role for SP came from immunohistochemical and other studies indicating the presence of large quantities of this peptide and its relevant receptors in the tissues of both brainstem dorsal vagal complex as well as in the gastrointestinal tract peripheral loci associated with emesis (see above). Indeed, early studies in dogs had indicated that SP is a potent emetogen at low doses (0.03−0.2 mg/kg) when administered i.v.[223] but not if injected i.p.[224] Species differences in the pharmacology of NK_1 receptor antagonists are known to be significant, and the ferret NK_1 receptors appear to be more "human-like". Since the ferret was the major animal model utilized for the development of 5-HT_3 antagonist antiemetics, it by default became the organism of choice for investigating the antiemetic potential of SP antagonists. In fact, the in vitro affinities of a wide range of NK_1 receptor antagonists for the ferret and human NK_1 receptor were shown to strongly ($r = 0.93$, $p = 0.0008$) correlate.[225] However, as with 5-HT (see section 4.5), systemically administered SP is not emetic in the ferret but can produce vomiting following central injection.[226,227] It is further surprising that research emphasis was solely focused on the emetic potential of NK_1 receptors, given that NK_1, NK_2, and NK_3 receptors as well as their endogenous ligands (SP, NKA, and NKB, respectively) are present in most emetic loci both in the brainstem and gastrointestinal tract. Even more intriguing, until very recently it was not established whether NK_1 receptor antagonists could completely prevent SP-induced emesis or whether NK_1 selective agonists are emetogenic. Indeed, recent evidence in the least shrew demonstrates that intraperitoneal administration not only of SP but also of brain-penetrable selective NK_1 agonists such as GR73632 induces vomiting in a dose-sensitive and NK_1 antagonist-sensitive manner.[92] Moreover, selective agonists of NK_2 and NK_3 receptors lacked emetogenicity, and their selective antagonists failed to prevent GR73632-induced vomiting in this species. To decipher the contribution of central/peripheral components of NK_1 receptor activation in the induced emesis, the antiemetic potential of several classes of NK_1 receptor blockers (e.g., CP99,994; CP122,721; GR203,040; GR205,171; HSP117; examples detailed in Figure 6C−F) were investigated against those emetogens unrelated to tachykinins, but whose site(s) of emetic action(s) in the chemotherapy-induced vomiting emetic reflex arc were known with some certainty. Thus, the ability of emetogens acting at the AP level (apomorphine, morphine, loperamide), via the vagus and splanchic nerves ($CuSO_4$), or mixed acting (cisplatin, cyclophosphamide, radiation), were shown to be blocked by NK_1 receptor antagonists.[165,228−231] Because of the ability of a given NK_1 receptor antagonist to reduce emesis caused by agents acting either centrally, peripherally, or mixed acting with a similar potency, it has been suggested that the antiemetic site of action of NK_1 receptor antagonists lies in the NTS or closely associated loci in the brainstem.[210]

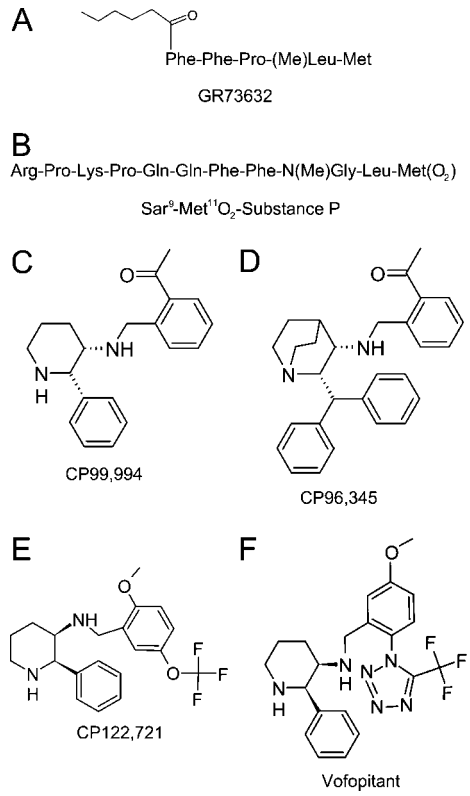

Figure 6. Structural relationships of emesis-modulating, NK_1 receptor binding tachykininergic compounds. (A) The brain-penetrant peptide derivative GR73632, a proemetic NK_1 receptor agonist. (B) The nonpenetrant peptide derivative Sar-Met-Substance P is also an NK_1 receptor agonist but lacks significant emetic activity due to its low penetrance. Brain penetrance was defined by the ability of these drugs to induce known centrally mediated behaviors such as ear scratches or head twitches. Compounds (C)−(F) are all NK_1 antagonists and show varying antiemetic efficacies: (C) CP99,994 was developed by replacing the quinuclidine ring structure with a piperidine ring, and the resuling compound was the first with both high NK_1 receptor affinity and high specificity. (D) CP96,345 was the first synthetic compound derived with high NK_1 receptor affinity, but the central quinuclidine ring structure caused a significant interaction with calcium channels. (E) CP122,721 was developed from CP99,994 using a trifluoromethoxy group to attempt to improve bioavailabilty. In testing for postoperative nausea and vomiting, CP122,721 was found to be on par with a similar NK_1 antagonist, aprepitant, and superior to ondansetron, a 5-HT_3 antagonist, in antiemetic efficacy. (F) Vofopitant is one of the newest NK_1 antagonists, and like aprepitant is heavily modified from the basic structure of CP99,994. Abbreviations: Arg, arginine; Gln, glutamine; Gly, glycine; Leu, leucine; Lys, lysine; Me, methyl; Met, methionine; Phe, phenylalanine; Pro, proline; Sar, sarcosine, *N*-methylglycine; Sar-Met Substance P, Sar^9-$Met^{11}O_2$-substance P.

Although direct injection of NK_1 antagonists (CP99,994 or HSP117) in the AP region has been shown to prevent emesis caused by peripheral administration of morphine or $CuSO_4$ in the ferret,[54] surgical removal of the AP does not interfere with the ability of CP99,994 to prevent $CuSO_4$-induced emesis in dogs.[232] This appears to be a paradox since vagally stimulated circuits inducing emesis in both species are similar.[53] More recent evidence may shed some light on this conundrum because intraperitoneal injection of the brain-penetrating NK_1 agonist GR73632 failed to cause Fos expression in the AP but induced emesis as well as Fos expression in both the NTS and DMNX of the least shrew.[92,93] A similar Fos expression pattern has been observed in vomiting least shrews receiving dopamine $D_{2/3}$ selective agonists[93] and in vomiting ferrets treated with loperamide.[113] In addition, microinjection of NK_1 antagonists in the vicinity of the NTS attenuates cisplatin induced emesis in ferrets.[229] These studies indicate that the site of initiation of SP-induced emesis in the brain resides in the NTS. In the decerebrate dog and ferret preparations, fictive retching can be produced by electrical stimulation of the vagus, and the induced effect can be prevented by direct injection of small doses of the NK_1 antagonist, GR205171, near the semicompact part of the nucleus ambiguus.[53,76,81] Neurons in the latter area are thought to receive inputs from the mNTS that in turn drive the central pattern generator for emesis, and thus NK_1 antagonists abolish the retching activity of neurons comprising the central pattern generator. Indeed, intraperitoneally administered GR73632 in least shrews induces both emesis and Fos expression in their central pattern generator. Additional evidence supporting a major central mechanism in the emetic/antiemetic actions of NK_1 receptor agonists/antagonists includes the following: (1) brain penetration is required for both emetic/antiemetic activity;[92,225] (2) intracranial injection of CNS penetrant or nonpenetrant NK_1 receptor antagonists prevents emesis produced by the peripheral administration of cisplatin;[226,229] and (3) there is strong correspondence in the rank order of potency between NK_1 receptor antagonists' ID_{50s} for their antiemetic activity in ferrets and their ability to suppress foot tappings induced by centrally injected NK_1 receptor selective agonists in gerbils.[225,233] Moreover, systemic administration of the CNS penetrable NK_1 receptor agonist GR73632 induces both emesis and scratching (a behavior analogous to foot tapping in gerbils) in the least shrew, and both behaviors are sensitive to NK_1 antagonists.[92]

Despite the above evidence, the question of a central and/or peripheral emetic action for tachykinins is clouded by inconsistent data from current animal models. As discussed earlier, SP is a potent emetogen in the dog, but only when administered i.v., while in ferrets the opposite effect has been noted. Moreover, intravenous administration of the NK_1 receptor antagonist RPR100893 was effective against cisplatin induced emesis in ferrets but did not reduce foot-tapping in gerbils.[225] These disparate findings suggest a peripheral component in addition to the proposed central action. Indeed, some peripherally acting NK_1 receptor antagonists such as sendide can prevent emesis produced by systemic administration of cisplatin[38] and reduce vagal afferent discharge produced by peripheral exposure to either SP or 5-HT.[38,44] The gastrointestinal tract can be another potential anatomical substrate of SP-induced emesis. NK_1 receptors and SP are present in vagal afferents, the ENS, and intestinal tissue, and can directly or indirectly stimulate intestinal motility (see earlier). Indeed, Fos-immunoreactivity is frequently noted in the ENS independent of emesis.[92] However, in vomiting least shrews, a modest but significant increase in Fos-immunoreactivity in the ENS was found. In addition, selective ablation of NK_1 receptors at a limited region of the least shrew small intestine by the targeted toxin SSP-saporin significantly attenuated the vomiting frequency and altered the quality of emesis produced by the NK_1 agonist GR73632. It also shifted the dose−response emetic curve of the agonist to the right without affecting the centrally mediated simultaneous induction of scratching behavior.[92] Likwise, central lesion of NK_1 receptors in the least shrew brainstem by the toxin caused a significant reduction in emesis frequency but also caused a trend toward reduction

of scratching behavior.[234] A combination of central and peripheral NK_1 receptor ablation did not further protect shrews from emesis but completely prevented the scratching behavior. Thus, it appears that peripheral NK_1 receptors have a facilitatory role in emesis, and the latter effect is probably secondary to, and possibly dependent on, the major central emetic component of NK_1 receptor activation. These findings, combined with the ability of SP to generate retroperistalsis[235] and to relax the lower esophageal sphincter (an event occurring in emesis) via NK_1 receptors,[236] are compelling evidence for involvement of peripheral NK_1 receptors in vomiting.

Since peripheral administration of SP causes emesis in several (e.g., dog, least shrews) but not all tested species (e.g., ferret), the source of SP involved in drug- or disease-induced emesis remains an enigma. The simplest scenario would be that cisplatin causes emesis following local release of SP within the brainstem, since central administration of peripherally ineffective doses of this agent has been reported to induce prolonged emesis in cats and pigeons.[169,170] A second potential source is the local release of mucosal SP in the gastrointestinal tract, which could activate emetic abdominal afferent NK_1 receptors to induce vomiting, a mechanism that is well-accepted for cisplatin induced 5-HT release (see earlier). Third, SP released from the gut may enter circulation and could act on NK_1 receptors in the AP region of the brainstem. This possibility appears unlikely, since NK_1 receptor activation via intraperitoneal administration of GR73632 failed to induce Fos expression in the AP.[92] On the other hand, it caused robust expression in the mNTS and less robust but significant expression in the DMNX,[92] suggesting that SP may enter the brainstem and directly act in the NTS and/or DMNX. Indeed, there is evidence that peripheral peptides can influence the brain directly in that systemically administered SP induces some centrally mediated effects in rodents.[237−239] Although unlikely to pass the blood−brain barrier under physiological conditions in sufficient quantities to cause emesis, SP may gain rapid entrance into the brain by a specific transport mechanism during chemotherapy-induced vomiting[240,241] through the AP or other circumventricular organs. The AP is located dorsal to the NTS, is outside the blood−brain and cerebrospinal fluid barriers, possesses active influx and efflux transport proteins,[242] and is sensitive to bloodborne chemicals.[243] Capillaries from the AP make vascular links with the NTS, which itself has fenestrated capillaries with high permeability.[244,245] Exogenously administered SP can be degraded rapidly in larger species[246,247] and particularly quickly in the least shrew, given that a large emetic dose (50 mg/kg, i.p.) attained maximal blood serum concentration within 5 min of injection and quickly declined toward basal levels within 5 more minutes.[92] Although maximal duodenal and jejunal tissue concentrations occurred within 5 min of injection, levels remained significantly above baseline for up to 15 min. Baseline concentrations of shrew brain tissue SP (8.5−55 pg/mg protein) were similar to those seen in rat brain (8−80 pg/mg protein).[248] Brainstem SP-tissue levels required a longer time both to reach maximum (15 min) and to decline to basal levels (also 15 min), which is likely to be due to relatively limited brain penetration through circumventricular organs. SP levels increased time-dependently in the brainstem but not in the frontal cortex, indicating SP entry was selectively confined to the dorsal vagal complex area, a result also seen following intracarotid SP injection in rats.[249] These findings correspond well with SP-induced emesis, in that the onset of emesis occurred within 1−2 min of injection, and the remaining episodes mainly occurred within 5 min of injection. These data support a rapid entry of SP into the brainstem without conflicting with published data[240−242,249] describing a specific, carrier-mediated transport mechanism for SP.

Since SP can be rapidly transported into the brain, the emetic effects of NK_1 receptor selective agonist analogues of SP whose varied compositions could alter their CNS-penetration were studied.[92] SarMet-SP is an undecapeptide, while ASMSP is a modified hexapeptide and GR73632 is a modified pentapeptide (see Figure 6A/B). The first two agonists caused no more than a couple of vomiting episodes, in 30−50% of tested shrews, and with few scratchings. None of these effects were statistically significant or dose-dependent. While there may be species differences involved because of NK_1 receptor differences, the inability of the latter compounds to significantly induce such behaviors appears to be due to poor penetration into the brainstem,[240] a mechanism supported by the fact that such agonists must be centrally administered to induce motor behaviors.[55,250] Indeed, species differences do not appear to affect the potency/efficacy of NK_1 receptor agonists.[250] Despite the likely low affinity for the SP active transport system, small amounts of the relatively nonpenetrant agonists could still pass into the brain at large doses, and this leakage could produce the weakly emetic response seen in some shrews. In addition to emesis, systemic administration of the brain-penetrating NK_1 receptor agonist GR73632 in the least shrew concomitantly produced scratching behavior in a dose-dependent manner. This behavior, analogous to mouse ear-scratching, is mediated centrally in both rodents and shrews via the stimulation of NK_1 or serotonergic 5-HT_{2A} receptors.[250−253] The cross-talk between the two neurotransmitter systems is demonstrable, in that blockade of NK_1 receptors prevents serotonergically induced scratching and head-twitching behavior in mice.[252] Foot tapping in gerbils, a similar centrally mediated behavior, can be induced by systemic GR73632 as well.[225] Since SP is metabolized primarily in the liver,[210] the amount reaching the brain from the periphery is limited under normal conditions. Three lines of evidence support the cardinal role of brain penetration in correlating NK_1 receptor activity and behavior: (1) systemic GR73632 caused dose-dependent emesis at less than 5% of the dose of systemic SP; (2) systemic SP failed to induce scratching; and (3) the measured basal SP concentration in shrew frontal cortex (a possible locus for scratching behavior)[253] actually decreased, indicating exogenous SP did not penetrate the telencephalon. One possible cause for a decrease in telencephalic SP is activation of inhibitory somatodendritic NK_1 receptors on serotonergic dorsal raphe neurons.[254] Indeed, the high levels of exogenous SP in the brainstem/midbrain activated these receptors and, thus, reduced endogenous SP levels in the frontal cortex via a negative feedback mechanism similar to that described for 5-HT.[255] In fact, NK_1 receptor antagonists seem to potentiate 5-HT tissue levels in the frontal cortex via such a mechanism.[256] One of the most impressive properties of NK_1 receptor antagonists is the nature of their broad-spectrum antiemetic efficacy in diverse animal models of emesis including ferrets, dogs, cats, piglets, pigeons, and house musk shrews. An extensive variety of emetogens acting via diverse mechanisms has been used to varying degrees in these animal species to investigate the antiemetic potential of different

classes of NK_1 receptor antagonists including CP99,994; GR205,171; L743,310; and PD154,075.[210,231] The tested emetic stimuli included apomorphine, morphine, loperamide, nicotine, $CuSO_4$, electrical stimulation, cisplatin, cyclophosphamide, radiation, ipecacuanha, phosphodiesterase inhibitors, ethanol, and resiniferatoxin. The latter reviews indicate that, although the various research teams have employed different experimental protocols including single versus multiple doses of NK_1 antagonists as well as different routes of administration and animal species, the general picture appears to be that brain penetration is often required for NK_1 antagonists to exhibit antiemetic efficacy, and unless emesis is prevented by 60−75%, most NK_1 receptor antagonists fail to reduce the latency to the first episode of retching and/or vomiting. In addition, NK_1 antagonists investigated in various animal species seem to abolish or attenuate emesis produced by the cited diverse emetic stimuli in a potent manner, including both the immediate and delayed phases of chemotherapy-induced vomiting (see later). More recent evidence indicates that NK_1 antagonists such as CP99,994 are also effective against prostaglandin E_2-induced vomiting in ferrets,[257] implying the possible utility of such antagonists for emesis caused by inflammatory GI disorders. Finally, relative to other tested emetic species, the house musk shrew is either nonresponsive (e.g., apomorphine) or relatively less sensitive to other emetogens such as morphine, loperamide, ipecacuanha, and cisplatin. Moreover, NK_1 antagonists are less potent in this species in abolishing emesis caused by nicotine, cisplatin, and motion, which suggests that NK_1 receptors in house musk shrews are different from other emetic species. Although NK_1 antagonists have not yet been evaluated against the discussed emetogens in the least shrew, this smaller species is much more sensitive to cisplatin[258] and vomits rapidly following administration of apomorphine as well as selective dopamine $D_{2/3}$ agonists.[118]

Some NK_1 antagonists that were efficacious in animals had to be modified prior to their introduction into the clinic. Figure 6 (C−F) provides samples of the modifications necessary for developing clinically effective NK_1 antagonists. For example, CP96,345 has very high affinity for Ca^{2+} channels, while others (e.g., CP122,721; L754,030) have very low or negligible affinity for the ion channel and, thus, better potential for clinical utility.[231] In addition, they should have a long half-life and be available in oral dosage form. Thus, CP99,994 was further chemically modified and superseded by CP122,721 and vofopitant. Studies determining the clinical potential of NK_1 receptor antagonists were approved in both the U.S.A. and Europe in 2003. Accumulated clinical trials indicate that NK_1 antagonists by themselves are not fully effective against cisplatin induced emesis in cancer patients, but they can potentiate the antiemetic efficacy of standard therapy (a 5-HT_3 antagonist plus dexamethasone) against both acute and delayed chemotherapy-induced vomiting.[210,231,259] On the other hand, NK_1 antagonists by themselves appear to demonstrate significant efficacy against emesis, but not nausea, in patients recovering from postoperative nausea and vomiting (PONV). Indeed, both aprepitant and CP122,721 were superior to ondansetron (a 5-HT_3 antagonist, see Figure 4C) for prevention of vomiting.[210,260] The combination of both antiemetics was not superior relative to each drug administered alone. However, the NK_1 antagonist GR205171 lacks efficacy against motion-induced nausea in humans and, thus, does not reflect NK_1 antagonist efficacy against motion-induced emesis in animals.[210,261−263] Thus, the broad-spectrum antiemetic activity of NK_1 receptor antagonists observed in animals may not fully translate into the clinic.

6. Cannabinoids and Vanilloids

Marijuana is the common name for the plant *Cannabis sativa*, and cannabis refers to the products of this plant. Owing to its psychotropic and medicinal effects, cannabis has been used throughout human history for different purposes. Basic research in the1960s and early 1970s led to the identification of the major psychotropic component of marijuana, (−)-*trans*-delta-9-tetrahydrocannabinol (Δ^9-THC).[264] In addition to phytocannabinoids, numerous chemicals with cannabimimetic activity have been synthesized. The mechanisms by which Δ^9-THC produces its cellular effects was revealed with the identification and cloning of at least two G-protein coupled receptors called cannabinoid CB_1 and CB_2.[265] Two splice variants of CB_1 receptors have been identified: CB_{1A}, which has an altered amino-terminal sequence, and CB_{1B}, which has an in-frame deletion of 33 amino acids at the amino terminus. While the CB_1 receptor is expressed in the neurons in the CNS, the CB_2 receptor is often localized in lymphoid tissues in the periphery. The presence and function of CB_2 receptors in brain neurons are controversial, although recent evidence suggests their presence on peripheral neurons. However, thus far only a few studies indicate the presence of CB_2-immunoreactivity or its mRNA expression on the neurons in the brain dorsal vagal complex subnuclei.[266] Cannabinoid CB_1 and CB_2 receptors share little sequence homology, and only 68% similarity occurs in their transmembrane domains, which are thought to contain the binding sites for cannabinoids. Δ^9-THC (chemically delineated in Figure 7A) and most synthetic cannabinoids (CP55,940; HU-210; WIN55,212-2; see Figure 7B) have similar affinities for the two receptors.[267] In more recent years, a number of selective CB_1 agonists (e.g., methanandamide, O-1812) and antagonists (e.g., SR141716A, AM251, AM281) have been synthesized (Figure 7C/D). Selective CB_2 agonists (e.g., JWH133, AM1241) and antagonists (e.g., SR144528, AM630) have also been discovered (Figure 7E/F). The cloning of CB_1 receptors was soon followed with identification of endogenous ligands generally referred to as endocannabinoids. To date at least two well-investigated endocannabinoids are recognized, *N*-arachidonoylethanolamide (also called anandamide) and 2-arachidonoylglycerol (2-AG), in both the brain and the gut (see also Figure 8). Several pathways exist for their formation and catabolism. Anandamide originates from the membrane phospholipid precursor, *N*-arachidonoylphosphatidylethanolamine, which is formed from the *N*-arachidonoylation of phosphatidylethanolamine via N-acyltransferases. *N*-arachidonoylphosphatidylethanolamine is then transformed by four possible alternative pathways, the most direct of which is catalyzed by *N*-acyl-phosphatidylethanolamine selective phospholipase D. When serving as an endocannabinoid, 2-AG is produced almost exclusively by the hydrolysis of diacylglycerols via sn-1-selective diacylglycerol lipases α and β. Following their cellular reuptake, anandamide is metabolized via fatty acid amide hydrolase (FAAH) and 2-AG via monoacylglycerol lipase (MAGL). 2-AG is also metabolized to some extent by other hydrolases as well as FAAH.[265] Thus far, only selective inhibitors of FAAH (e.g., URB-597, arachidonoylserotonin, SA7) have been developed; they act as indirect agonists and thus can produce cannabimimetic

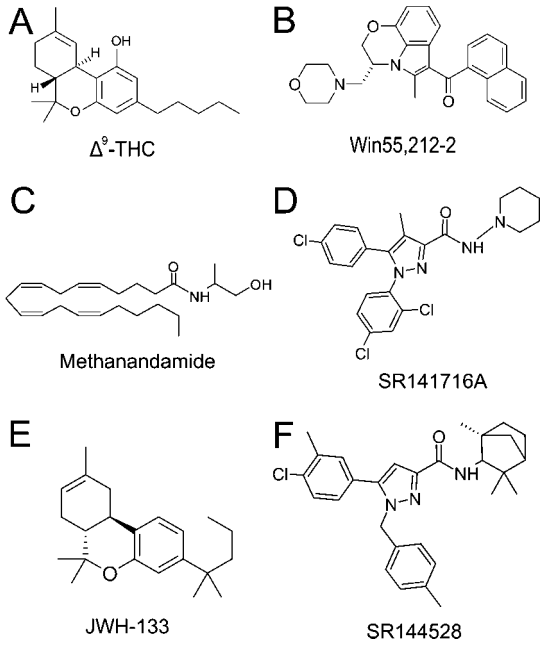

Figure 7. Structural relationships of cannabinoid receptor-binding compounds. Cannabinoid receptors are frequently inhibitory heteroceptors, and as such, agonists of cannabinoid receptors are antiemetic. Cannabinoid receptor antagonists are either emetically neutral or, at high doses, can be proemetic. (A) Delta-9-tetrahydrocannabinol, the primary psychoactive constituent of *Cannabis sativa* and nonspecific cannabinoid receptor agonist. Δ^9-THC demonstrates antiemetic activity in vivo, primarily through binding of CB_1 receptors, although there may be some CB_2-related antiemetic activity as well (detailed in the text). (B) Win55,212-2 is a synthetic cannabinoid derived from a heavily modified aminoalkylindole base structure, with higher affinity for CB_1 receptors than Δ^9-THC. (C) Methanandamide, a synthetic, arachidonic acid-based cannabinoid agonist with reasonably high specificity for CB_1 receptors. The chemical is derived from the endocannabinoid *N*-arachidonoylethanolamide (anandamide, see Figure 8). (D) SR141716A is a highly specific CB_1 receptor antagonist. At high doses, it can induce vomiting. (E) JWH-133 is a specific CB_2 receptor agonist. It does not have psychoactive or antiemetic activity in vivo. (F) SR144528 is a specific CB_2 receptor antagonist. As with the agonist JWH-133, it has no psychoactive activity in vivo. Abbreviations: Δ^9-THC, Delta-9-tetrahydrocannabinol.

activity. Although the endocannabinoid cellular reuptake mechanism is yet to be fully characterized and still remains controversial, this reuptake system appears to also mediate the release of newly synthesized anandamide and 2-AG.[265] Increasingly, more selective inhibitors of this reuptake process are being developed including OMDM-1 and UCM-707. These compounds also act as indirect agonists and produce endocannabinoid-like effects. Under certain conditions, both anandamide and 2-AG might become substrates for COX-2 and will give rise to the corresponding hydroperoxy derivatives, which can then be converted to prostaglandin ethanolamides (prostamides) and prostaglandin glycerol esters, respectively, by various prostaglandin synthases. The latter metabolites are inactive at cannabinoid receptors. Anandamide also interacts with several noncannabinoid receptors, including the transient receptor potential vanilloid subtype 1 (TRPV1) receptor, to which it binds at an intracellular site. Although anandamide is thought to be the major endogenous ligand for TRPV1 receptors, other potential endogenous candidates include several products of lipoxygenases such as 12-(*S*)-HPETE, 15-(*S*)-HPETE, LTB_4, and *N*-arachidonoyldopamine.[268] Both endocannabinoids may also activate an orphan G-protein-coupled receptor, GPR5.[265] However, most often the effects of cannabinoids have been studied through CB_1 and CB_2 molecular targets. Anandamide has the highest affinity, whereas 2-AG has the greatest efficacy for cannabinoid CB_1 and CB_2 receptors. Retrograde signaling is an important aspect of cannabinoid function where, upon postsynaptic stimulation, endocannabinoids are synthesized on demand in postsynaptic neurons and diffuse back to presynaptic nerve terminals and stimulate CB_1 receptors to inhibit neurotransmitter release in the CNS. Phytocannabinoids as well as synthetic cannabinoids act as agonist antiemetics via cannabinoid CB_1 receptors, whereas endocannabinoids possess pro- and antiemetic actions.[269] Although most published studies exclude a role for CB_2 receptors in emesis, a recent study indicates a minor role for this receptor in vomiting.[266] Anandamide may also provide protection against emesis via its endovanilloid agonist activity through the activation of TRPV1 receptors.[270] Both cannabinoid CB_1 and CB_2 receptors, as well as TRPV1 receptors, can affect gastrointestinal tract function and emesis as described below.

6.1. Dorsal Vagal Complex

Significant concentrations of both anandamide and 2-AG are found in different parts of the mammalian brain. 2-AG tissue levels are approximately 1 order of magnitude greater than anandamide, with particularly high levels of both endocannabinoids in the brainstem.[266,271] Tissue levels of endocannabinoids in specific subnuclei of the dorsal vagal complex have not yet been investigated except for the NTS, which contains significant levels of anandamide.[272] Immunohistochemical studies in the ferret brainstem have revealed dense levels of CB_1-immunoreactivity in the mNTS and DMNX and moderate staining in the area postrema.[50,273] Furthermore, CB_1-immunoreactive terminals surrounded FAAH immunoreactive cell bodies in the ferret DMNX. Immunohistochemical, autoradiographic, brain homogenate radioligand- and GTPγS-binding studies in the least shrew brainstem also indicate a similar distribution of CB_1 receptors in the dorsal vagal complex, with CB_1 receptors being specially dense in the NTS with more sparse levels in the DMNX and AP regions.[93,274] Some punctate CB_1-immunoreactivity (putative terminal) labeling in the least shrew was colocalized with punctate immunoreactivity for 5-HT and/or SP neuronal terminals in the NTS.[93] CB_1-immunoreactivity and/or mRNA expression is also found in the brainstem subnuclei of several species including humans.[275,276] Cannabinoids may act at three possible sites in the brainstem to reduce cisplatin induced emesis:

(1) At presynaptic CB_1 receptors to inhibit neurotransmitter release from the vagal afferent terminals, thus preventing afferent transmission. Therefore, a reduction in Fos-immunoreactivity would be expected in neurons downstream of synaptic connections. Indeed, this is the case since Δ^9-THC reduces cisplatin induced Fos-immunoreactivity during acute emesis in both the ferret and least shrew NTS and DMNX in a CB_1 antagonist-sensitive manner.[93,277]

(2) On CB_1 receptors present on the terminals of inhibitory interneurons within the NTS that receive inputs from vagal afferents. These inhibitory interneurons probably reduce the activity of excitatory NTS neurons that project to the DMNX, which could lead to suppression of visceral motor responses.[277,278]

(3) On CB_1 receptors present on the terminals of NTS neurons that project to the DMNX or the AP. Indeed, the enhanced Fos activity in both the ferret and least shrew NTS

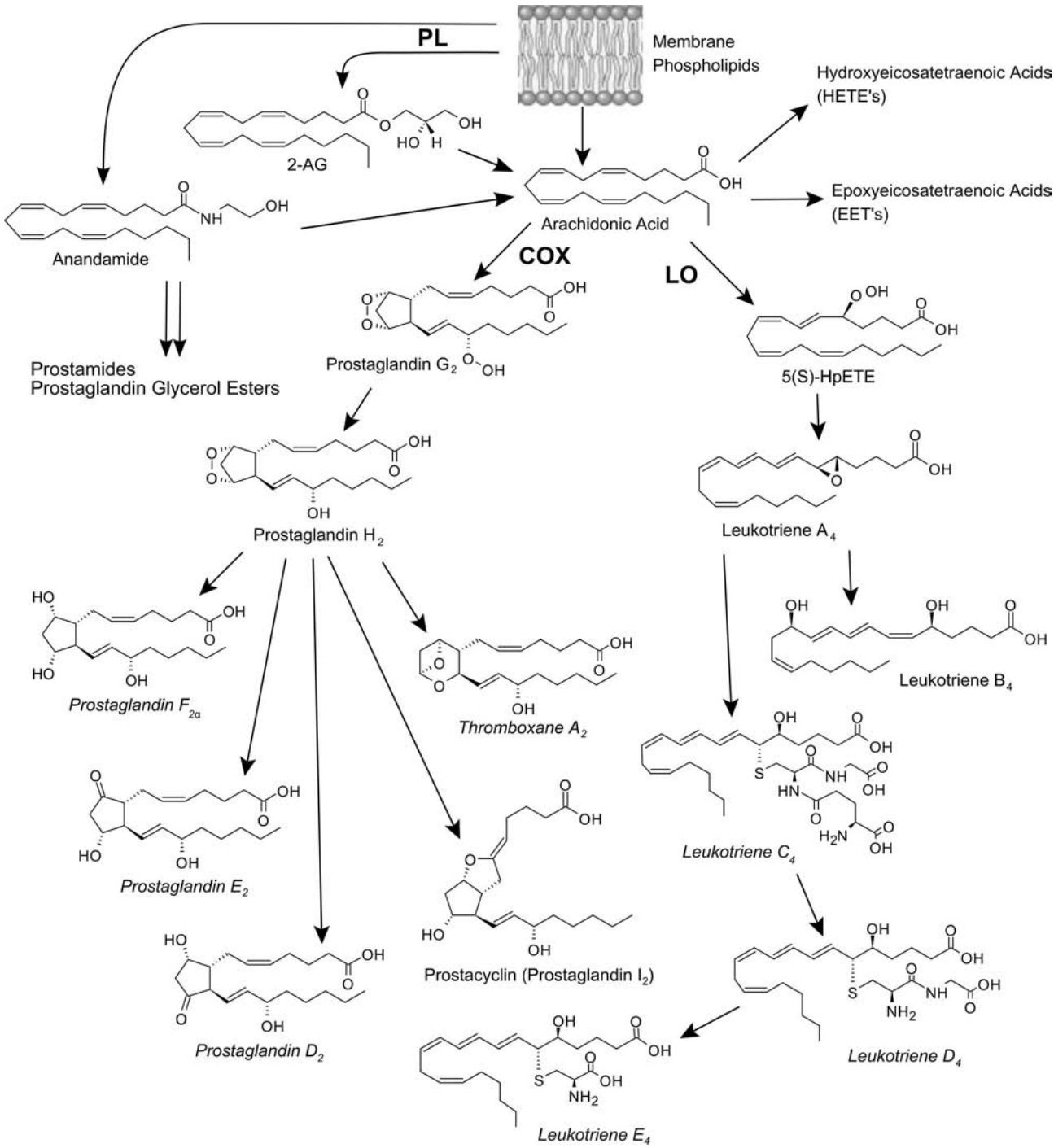

Figure 8. Biosynthesis of eicosanoids and endocannabinoids. In both cases, membrane phospholipids are enzymatically metabolized to produce the lipid-based eicosanoids and endocannabinoids. The endocannabinoids, anandamide and 2-arachidonoylglycerol, are produced from phospholipids via phospholipase (PL) activity. Both species can be metabolized back to arachidonic acid, or modified further to produce prostamides or prostaglandin glycerol esters. The other eicosanoids are produced via several different enzymes acting upon arachidonic acid, which is itself produced enzymatically from phospholipids. Several "families" of eicosanoids are produced from arachidonic acid, depending on the initial enzymatic pathway. The main metabolites of two families with known emetogenic variants are depicted. The prostanoid (prostaglandin-related) family is produced via cyclooxygenase activity (COX), and the leukotriene family is produced via lipoxygenase (LO) activity. Dozens of related metabolites exist within each of these families, so for clarity only major emesis-related metabolites and their precursors are shown in detail. The cysteinyl leukotrienes, which have a glutathione-derived moiety that may allow cross-reactivity with cisplatin transporting proteins, are the leukotrienes C_4, D_4, and E_4 (italicized). Abbreviations: 2-AG, 2-arachidonoylglycerol; COX, cyclooxygenase; LO, lipoxygenase; PL, phospholipase.

following acute cisplatin induced vomiting was reduced by Δ^9-THC in a CB_1 receptor-dependent fashion.[93,277]

The downstream target of this activation, the DMNX, also exhibited decreased Fos activity following Δ^9-THC pretreatment. The large reduction in Fos-immunoreactivity in the area postrema of cisplatin exposed ferrets and least shrews following Δ^9-THC pretreatment is probably due to either a modulatory input to the AP from the NTS regulated by CB_1 receptors and/or Δ^9-THC-induced reductions in the release of bloodborne emetogens such as 5-HT, SP, or prostaglandins.

In the least shrew, labeling for CB_2 was practically nonexistent in the dorsal vagal complex itself, with the

exception of one or two elements morphologically indicative of vascular walls. Also, the choroid plexus and the surface of the brainstem demonstrated moderate levels of CB_2 immunoreactivity. However, both the ferret and rat AP and DMNX appear to express CB_2 mRNA, and CB_2-immunoreactivity was shown to also occur in the ferret DMNX.[266] Using anandamide and 2-AG as well as indirect agonists (uptake inhibitors or catabolic inhibitors) combined with selective $CB_{1/2}$ antagonists, the latter authors have indicated that CB_2 receptor activation may also have an antiemetic role against morphine-6-glucuronide induced vomiting.[266] However, not only do direct-acting and selective synthetic CB_2 agonists (AM1241 or JWH 133) fail to block the induced emesis in the latter study, previous publications of these authors[270,273,277] and numerous other studies have discounted a role for CB_2 receptors against a variety of emetogens (see later).

Endovanilloid TRPV1 receptor-immunoreactivity in the ferret brainstem also appears to be most abundant in the NTS, with less labeling in the DMNX and AP.[270] Within the NTS, these receptors were most abundant in the subnucleus gelatinosus, the medial subnucleus, and the solitary tract itself, with labeling mostly localized to fibers and terminals. There appears to be a high degree of colocalization of CB_1 and TRPV1 receptors in dorsal and medial nuclei of the NTS and in motor neurons of the DMNX, as well as in a few scattered neurons of the AP. This colocalization may have functional importance in the antiemetic efficacy of hybrid agonists (e.g., arvanil) stimulating both receptors. Resiniferatoxin obtained from *Euphorbia* sp., is an ultrapotent agonist of TRPV1 receptors. It is an analogue of the sensory neurotoxin capsaicin, which itself is the hot ingredient of chili peppers. The mechanism and site of antiemetic action of resiniferatoxin has been suggested to be stimulation of TRPV1 receptors in the terminal portion of capsaicin-sensitive, SP-containing emetic vagal afferents in the mNTS. SP is postulated to be the emetic neurotransmitter in the synapse between these vagal afferent terminals and the neurons of the mNTS that drive the central pattern generator for vomiting.[279]

6.2. Vagal Afferents

Immunohistochemical evidence indicates that CB_1-immunoreactivity is present on the cell bodies of vagal afferent neurons in the ferret, rat, and human nodose ganglion, and that CB_1 receptor is largely transported to the peripheral terminals rather than to central terminals.[50,280] Cannabinoids can affect emesis not only by modulation of vagal afferent activity to the dorsal vagal complex nuclei (see above) but also via vagal efferents, since gastric motor inhibition caused by systemic Δ^9-THC can be abolished by vagatomy, and Δ^9-THC applied to the dorsal surface of the medulla mimics the effect of intravenously administered Δ^9-THC.[281] Vagal afferents have their cell bodies in the DMNX and project to both submucosal and myenteric plexi and their terminals contain CB_1 receptors.[282] The main neurotransmitter in these nerves is acetylcholine, which influences motility, secretion, and blood flow by interacting with enteric nerves. Thus, cannabinoids may also exert their antisecretory and antimotility actions at this level via the activation of presynaptic CB_1 receptors. Currently, the presence of CB_2 receptor markers has not been confirmed in vagal afferents. However, CB_2 receptor-immunoreactivity is present on peripheral sensory neurons and colocalizes with both CB_1 and TRPV1 receptors, as well as modulating TRPV1 sensitivity via cAMP depletion.[283] Thus, if the CB_2 receptor is also present on vagal afferents and exhibits similar colocalization, then vagal activity could be modulated by CB_2 receptor stimulation. Stimulation of TRPV1 receptors on vagal afferents by either capsaicin or resiniferatoxin is traditionally thought to involve an initial excitatory effect that leads to neurotransmitter release in the NTS (e.g., SP) and emesis, followed by desensitization and a refractory period (with possible depletion of SP in the NTS or other dorsal vagal complex emetic nuclei), where animals would not respond to different emetic stimuli including electrical stimulation of the vagus,[279] intragastric $CuSO_4$, radiation, loperamide, and cisplatin in different species.[284−286] Indeed, immunohistochemical, molecular, and electrophysiological evidence has confirmed the presence of TRPV1 receptors in the gastrointestinal tract vagal afferent neurons.[287,288] Thus, TRPV1 agonists such as resiniferatoxin also appear to be potent and broad-spectrum antiemetics.

6.3. Enteric Nervous System

To date, the release of endocannabinoids in the ENS has not been well-investigated, but the ENS appears to be an important cannabinoid source for the gastrointestinal tract. In addition, both anandamide and 2-AG can be released from non-neuronal sites such as endothelial cells.[282] Since endocannabinoids are not released from vascular smooth muscle, it is unlikely that gastrointestinal smooth muscles are a source of endocannabinoids. Immunohistochemical and mRNA expression studies indicate that enzymes for the degradation of both 2-AG and anandamide (MAGL and FAAH, respectively) are present in the cell bodies and nerve fibers of myenteric neurons in the small intestine.[282,289] MAGL enzyme activity was highest in the rat duodenum and tended to decrease along the gut with the lowest levels in the distal colon. CB_1-immunoreactivity colocalizes with specific markers of (1) all cholinergic neurons (e.g., choline acetyltransferase) in the guinea pig, rat, and porcine myenteric plexi, (2) most excitatory motor neurons (e.g., calretinin) to longitudinal muscles, (3) ascending excitatory cholinergic interneurons (e.g., calretinin), (4) some small population of SP neurons, and (5) intrinsic primary afferent neurons (e.g., calbindin).[282] The predominant action of cannabinoids on motor neurons appears to be CB_1 receptor-mediated presynaptic inhibition of gastrointestinal transit by attenuating transmitter release from excitatory motor neurons. Furthermore, it appears that neither CB_1 receptors nor MAGL are colocalized with nitric oxide synthase-containing inhibitory neurons.[282] Thus, cannabinoid agonists are potent inhibitors of gastrointestinal tract contractility, and inhibition of motility from stomach to colon occurs primarily via activation of enteric CB_1 and not CB_2 receptors under physiological conditions.[290] This reduction in peristalsis may contribute to the peripheral antiemetic component of cannabinoids.[168] On the other hand, in the lower esophageal sphincter, cannabinoids inhibit relaxation via the brainstem, and this effect may also in part account for their antiemetic properties.[50,291]

More recent molecular and immunohistochemical evidence indicate that CB_2 receptor mRNA and protein are also present in the majority of myenteric neurons along the gastrointestinal tract but not on those expressing nitric oxide synthase.[292] CB_2 receptors do not appear to affect gut motility under physiological circumstances, but potentially regulate motility in pathophysiological states. Indeed, functional studies

indicate that the CB_2 agonist JWH133 was unable to affect the electrically evoked twitch response of the rat ileum under physiological conditions but inhibited the enhanced contractile response in lipopolysaccharide-pretreated animals in a dose-dependent and CB_2 antagonist-sensitive manner. CB_2 receptors may also regulate tissue response to gut inflammation by either direct suppression of pro-inflammatory mediators or by affecting the response of smooth muscle to such stimuli.[293] In addition, in hyperalgesic states both CB_1 and CB_2 selective agonists were more potent in attenuating visceral pain produced in rodents by graded colorectal distension.[293,294] Indeed, the analgesic effects of CB_2 receptor agonism in somatic nerve pathways have been well-described, as has CB_2-mediated inhibition of visceral nerves supplying the gastrointestinal tract.[295] TRPV1-immunoreactivity has been identified in nerves within myenteric ganglia and interganglionic fiber tracts throughout the gastrointestinal tract. TRPV1-expressing nerves have also been observed within the (1) muscle layers, (2) blood vessels in the gastrointestinal wall, and (3) mucosa.[268,296] In addition, TRPV1-immunoreactivity is expressed by primary afferent neurons innervating the gastrointestinal tract. Activation of TRPV1-expressing cholinergic neurons in the myenteric plexi apparently contributes to the development of enhanced intestinal motility and secretion. Indeed, intraluminal administration of anandamide causes inflammation similar to *Clostridium difficile* toxin A in the rat ileum in a capsazepine (a TRPV1 antagonist)-sensitive manner that is not affected by cannabinoid $CB_{1/2}$ antagonists.[297] Cholinergic secretomotor neurons also contain neuropeptide Y (NPY), while noncholinergic secretomotor nerves contain vasoactive intestinal peptide (VIP). These nerves project to the mucosa, regulate water and electrolyte levels, and are controlled through local reflexes and the CNS via sympathetic nerves. They also project to submucosal blood vessels and control blood flow. CB_1-immunoreactivity colocalizes with all VIP-containing neurons and the majority of NPY-containing neurons in the guinea pig ileum. However, CB_1-immunoreactive receptors do not colocalize with VIP in the porcine myenteric and submucosal plexi. Activation of CB_1 receptors on cholinergic neurons in the submucosal plexus limits cholinergic nerve-mediated secretion, while blockade of these receptors leads to fluid accumulation and diarrhea-like symptoms.[282,294] On the other hand, CB_2 antagonists are devoid of such symptoms.

6.4. Gastrointestinal Tissue

As discussed earlier, intestinal smooth muscle tissue does not produce endocannabinoids, and thus reports concerning intestinal tissue levels of 2-AG and anandamide reflect neuronal and non-neuronal sources such as vascular endothelial cells, intestinal epithelial cells, platelets, and macrophages.[282,289] Relatively large amounts of 2-AG and anadamide (44 nmol/g of tissue and 36 pmol/g of tissue, respectively) are present in the small intestine of mice.[298,299] Indeed, mouse intestinal tissue concentration of 2-AG exceeds that of liver, spleen, lungs, and kidneys by 33−55 times, and of various brain regions by 3−20-fold.[298] On the other hand, the level of anandamide in both the CNS and peripheral tissues can be similar to, lower than, or greater than that present in the mouse small intestine. High intestinal levels of both 2-AG and anadamide are also present in the least shrew.[300] Regional differences in endocannabinoid levels in the gastrointestinal tract occur with 2-AG being higher in the ileum than the colon and anandamide being considerably higher in the colon than the ileum, which may reflect a difference in the functional activity of these endocannabinoids in the small and large intestine. In addition, the main degradation enzymes for anandamide and 2-AG are also highly concentrated in the intestine. Stress and pathophysiologic states can affect gut endocannabinoid levels since: (1) Hunger increases anandamide levels in the small intestine. (2) Anandamide tissue levels increase in the rat and mouse models of colitis and in mucosal biopsy samples obtained from patients with inflammatory bowel disease. (3) Cisplatin tends to reduce 2-AG and anandamide intestinal tissue levels in least shrews.[51,298−300] The presence of CB_1 receptor or its markers has been confirmed in the entire gastrointestinal tract on neurons supplying the stomach to the colon of several emetic and nonemetic species including humans.[282,298,299] However, CB_1 receptors are differentially distributed along the length of the gastrointestinal tract, with stomach and colon being highly enriched with these receptors. Although the discussed effects of endocannabinoids on gastrointestinal tract motility are thought to be of neural origin since cannabinoid CB_1 stimulation does not directly suppress smooth muscle activity, more recent evidence indicates that the major metabolic enzyme for 2-AG degradation (MAGL), as well as CB_1 receptors, are also highly expressed in the gastrointestinal tract epithelium.[289,294]

6.5. Cannabinoids and Emesis

Unlike the development of most drugs, clinical research on the antiemetic potential of Δ^9-THC and other cannabinoids against chemotherapy-induced vomiting has often preceded the necessary basic research in animal models of emesis.[301] The clinical trials were generally based on the past anecdotal information from India and the Middle East that cannabis products can be useful in nausea, vomiting, and diarrhea, and in particular on reports of decreased emesis exhibited by younger patients who used marijuana while receiving chemotherapy. At least six different cannabinoids have been evaluated for their antiemetic potential in over 40 clinical trials involving phytocannabinoids such as Δ^9-THC and Δ^8-THC, as well as synthetic cannabinoids such as nabilone and levonantradol.[301−303] The general conclusion appears to be that cannabinoids have a better antiemetic efficacy than dopamine D_2 antagonist antiemetics (such as prochlorperazine, chlorpromazine, haloperidol, or metchlopramide) against the frequency of vomiting episodes and severity of nausea caused by chemotherapy-induced vomiting. Testing of a combination of a cannabinoid agonist with a D_2 antagonist versus each compound alone, has shown either no enhancement or a greater antiemetic efficacy in cancer patients receiving chemotherapy.[301] However, the dopamine D_2 antagonists used in these early clinical trials are generally not very selective. In a recent animal study, the more selective D_2 antagonist sulpride failed to potentiate the antiemetic efficacy of Δ^9-THC against high-dose cisplatin induced emesis in the least shrew.[304] Although the advent of 5-HT_3 receptor antagonists in the 1980s led to the demise of further cannabinoid antiemetic research in the clinic, the discovery of the discussed cannabinoid receptors and their endogenous ligands combined with the introduction of new animal models of emesis, have paved the way for a renaissance in the field. The first published paper providing evidence that the antiemetic effect of cannabinoids is mediated via the activation of CB_1 (and not CB_2) receptors

was in the least shrew.[305] We envisaged that since cannabinoid CB_1 receptor activation prevents emesis, its antagonism should cause vomiting. Indeed, large doses (10−20 mg/kg, i.p.) of SR141716A (and not the CB_2 antagonist SR144528) produced emesis in a dose-dependent manner, and the response was blocked by both Δ^9-THC and synthetic cannabinoids. Although the induced vomiting can be attributed to the inverse agonist nature of SR141716A, this agent causes the release of large amounts of emetogenic monoamines such as DA and 5-HT in the shrew brainstem and rat hypothalamus.[306,307] SR141716A administration also causes nausea or emesis in 4−14% of overweight patients who had received low doses (0.05−0.2 mg/kg) of the antagonist.[308] Likewise, SR141716A has been reported to induce vomiting in Δ^9-THC-tolerant dogs,[309] while administration of small doses of another CB_1 antagonist (AM251) was shown to potentiate morphine-6-glucuronide induced emesis.[273] Since activation of presynaptic CB_1 receptors inhibit neurotransmitter release, this could be one mechanism by which cannabinoid agonists alleviate emesis. Δ^9-THC and related cannabinoids (WIN55-212-2; CP55,994; HU-210) possess broad-spectrum antiemetic activity in several animal models of emesis, in a CB_1 receptor antagonist-sensitive manner, and against diverse emetogens including: (1) acute-phase emesis by cisplatin,[274,277,310−314] (2) delayed-phase cisplatin,[93,315] (3) 5-HT precursor (5-hydroxytryptophan), serotonergic selective (2-methylserotonin), and nonselective (5-HT) 5-HT_3 receptor agonists,[168] (4) DA precursor (L-DOPA) and dopaminergic D_2/D_3 selective (quinpirole, quinelorane, or 7-(OH) DPAT) and nonselective (apomorphine) agonists,[111,316] (5) the endocannabinoid 2-AG,[269] (6) arachidonic acid,[269] (7) radiation,[317] (8) SP,[318] (9) morphine or morphine-6-glucuronide,[273,319] (10) motion,[320] and (11) *Staphylococcus* enterotoxin.[321] Cannabinoids' broad-spectrum antiemetic properties against emetogens in general, and their effectiveness against both acute- and delayed-phase chemotherapy-induced vomiting in animals[93] and cancer patients,[315] propels this class of agonist antiemetics to the forefront of research in terms of both mechanisms of action as well as sites of action. Indeed, as discussed earlier, cannabinoids can abolish emesis via both peripheral and central mechanisms at the myenteric plexus level, at the level of vagal afferents and efferents, and at the brainstem level. This is reflected by our findings that, while low doses of Δ^9-THC (<0.1 mg/kg, i.p.) can completely prevent the centrally mediated head-twitch and ear-scratch behaviors produced by the brain-penetrating 5-HT_3 agonist, 2-methyl-5-HT, in a one-phase fashion in the least shrew, Δ^9-THC pretreatment concomitantly attenuated the induced vomiting in a biphasic manner, with the central component being inhibited at doses less than 0.1 mg/kg, while the complete abolition of the peripheral emetic component required more than 20 mg/kg Δ^9-THC.[168] Likewise, Δ^9-THC was 4 times more potent in protecting shrews from centrally mediated 5-HTP-induced emesis in the presence of the peripheral decarboxylase inhibitor carbidopa, where conversion of 5-HTP to 5-HT in the periphery was prevented. Indeed, in the absence of carbidopa, 5-HTP-induced emesis was inhibited by Δ^9-THC in a biphasic manner, while inclusion of carbidopa transformed the Δ^9-THC-induced dose-reponse inhibition curve to a single central component in which Δ^9-THC's antiemetic efficacy was apparent at low doses. Further, Δ^9-THC prevents peripherally mediated 5-HT-induced emesis at high doses via a single component. Although in a single-dose combination study the antiemetic efficacy of ondansetron (a 5-HT_3 antagonist) plus dexamethasone was not potentiated by Δ^9-THC in patients receiving chemotherapy,[322] dose−response studies indicate that low doses of either ondansetron or tropisetron do potentiate the antiemetic efficacy of low but not high doses of Δ^9-THC against cisplatin induced emesis in both the least and house musk shrews.[312,323] Although generally disappointing, the lack of persistent additive or synergistic antiemetic action across doses when a cannabinoid agonist is combined with a 5-HT_3 antagonist is not surprising. There is likely to be a large overlap in the mechanisms with which these drugs block emesis, which would prevent the hoped-for enhanced antiemetic effect. For example, the mechanism of CB_1 receptor antiemetic agonists, as stated above, likely relies on presynaptic inhibition. This CB_1-mediated inhibition (e.g., in the dorsal vagal complex or GI nerve plexi) could reduce activity generated by postsynaptic, tropisetron-sensitive, 5-HT_3 receptor-containing neurons, or by presynaptic terminals that might colocalize these 5-HT_3 receptors.[324] In fact, there is also evidence that cannabinoids can directly modulate 5-HT_3 receptors allosterically.[325,326] If this direct cross-talk is also part of the mechanism of cannabinoid-mediated antiemesis, any potential additive effect may be dampened by interference from 5-HT_3 antagonist binding. The slight enhancement of antiemetic ability by low doses of Δ^9-THC in combination with low doses of tropisetron would result from incomplete receptor occupancy by either or both drugs, or possibly by incomplete anatomical overlap of cannabinoid and 5-HT_3 receptors.

Although not as well-investigated, DA may also possess central as well as peripheral components of emetic actions (see section 3.5). Using a similar logic to the biphasic nature of 5-HT inhibition, diverse cannabinoids seem to prevent emesis caused either by the DA precursor L-DOPA (with or without carbidopa), or by brain-penetrating direct-acting D_2/D_3 selective agonists, through a single-component inhibition curve that may indicate the importance of a solitary site of antiemetic action of cannabinoids for DA-induced emesis.[111,168] However, this requires further confirmation. Δ^9-THC also inhibits the ability of another identified emetogenic transmitter of chemotherapy-induced vomiting, SP, in a dose-dependent manner in the least shrew.[318] Unlike the well-accepted dogma that SP is mainly involved during the delayed chemotherapy-induced vomiting phase, both recent studies in the least shrew brainstem and jejunum[258] and clinical data in cancer patient's plasma[7,8] have shown that large amounts of this peptide are released during both phases of cisplatin induced vomiting. Furthermore, as already discussed, Δ^9-THC not only inhibits SP-induced emesis in a dose-dependent manner via CB_1 receptors but also blocks both the immediate and delayed phases of emesis caused by cisplatin.[93,315] Finally, addition of the anti-inflammatory glucocorticoid dexamethasone seems to add to the antiemetic potential of cannabinoids in cancer patients receiving chemotherapy.[3] However, a recent multidose−response combination study against high-dose cisplatin in the least shrew failed to show a dose-dependent interaction.[323] In the case of dexamethasone, emetic behavior would be mediated "downstream" from the presynaptic events modulated by CB_1 receptors. Postsynaptic second-messenger systems, including the prostanoid producing arachidonic acid metabolic pathways, would provide an interface through which dexamethasone and cannabinoid mediated systems would overlap.

The net effect in this case would be cannabinoid mediated inhibition, or lack of stimulation, of neurons whose downstream antiemetic effector mechanisms were already inhibited, preventing the proposed enhancement of antiemetic activity by the combined drug regimen.

From the above discussion regarding the antiemetic efficacy of phyto- and synthetic cannabinoids against chemotherapy-induced vomiting, combined with the knowledge of the emetogenic potential of CB_1 receptor antagonists, we hypothesized that endocannabinoids should attenuate cisplatin induced vomiting. However, exogenous administration of either anandamide or 2-AG in the least shrew lacked efficacy against cisplatin's vomiting (Darmani, unpublished findings). On the other hand, cisplatin caused dose- and time-dependent increases in endogenous basal levels of 2-AG but not anandamide in the least shrew brain, while concomitantly reducing intestinal tissue concentrations of both endocannabinoids.[300] Moreover, intraperitoneal injection of 2-AG was shown to cause dose-dependent emesis at low doses (1−2.5 mg/kg, i.p.) in a CB_1 antagonist-sensitive manner, whereas anandamide was emetogenic at 10 mg/kg but not at lower or higher doses, while its more stable analogue methanandamide lacked emetic activity.[269] We attributed the emetogenicity of 2-AG to its rapid metabolism since its major metabolite (arachidonic acid) is also a potent vomit inducer, and the emetic capacity of both emetogens can be prevented in the least shrew by the cyclooxygenase inhibitor, indomethacin. Not surprisingly, indomethacin has also been shown to attenuate cisplatin induced emesis in piglets.[327] Furthermore, pretreatment with either anandamide, methanandamide, phyto-, or synthetic cannabinoids prevents the ability of 2-AG to cause emesis in the least shrew.[269] Indirect agonists of the endocannabinoid system, such as selective inhibitors of FAAH (arachidonoylserotonin or URB597) or selective reuptake inhibitors (OMDM1 or VDM11) have also been tested in the least shrew against several emetogens (cisplatin, apomorphine, or 2-AG), but none of them had consistent antiemetic activity.[269] Indeed, some of these (arachidonoylserotonin, URB597, and OMDM1) caused vomiting at larger doses (>10 mg/kg, i.p.) by themselves. There appears to be some species differences in the emetic/antiemetic efficacy of endocannabinoids and their indirect agonists. For example, in the ferret, methanandamide causes retching but not vomiting,[273] while anandamide, 2-AG, VDM11, and URB5973 lacked emetic/retching activity at 2−3 mg/kg doses. The inability of the ferret to vomit in response to intraperitoneal injection of 2-AG may not be surprising, since neither 5-HT nor SP induce emesis in this species via the peripheral routes (see sections 4 and 5). Furthermore, these compounds appear to prevent vomiting caused by morphine-6-glucuronide in the ferret via activation of both CB_1 and CB_2 receptors.[266] However, previous reports from the latter authors as well as numerous other publications have discounted a direct role for CB_2 receptors in emesis.

Not only is anandamide an endocannabinoid, it also behaves as an endovanilloid and may produce its antiemetic activity via stimulation of both cannabinoid CB_1 and vanilloid TRPV1 receptors. Indeed, antiemetic actions of anandamide and other hybrid compounds such as arvanil and NADA against morphine-6-glucuronide induced vomiting can be reversed in ferrets by either CB_1 or TRPV1 antagonist pretreatment.[270] As discussed earlier, potent and selective agonists of TRPV1 receptors such as resiniferatoxin exhibit an initial emetic activity by themselves, and subsequently antiemetic efficacy when tested against a diverse array of emetogens. Gastrointestinal resiniferatoxin-sensitive vagal afferent C-fiber terminals contain SP, as well as TRPV1 receptors, and stimulation of these receptors seems to release SP to activate neurons of the mNTS (see section 5.5). These neurons in turn drive the central pattern generator to induce vomiting. However, the enhanced firing in the mNTS gradually subsides and the response of these neurons to stimulation of abdominal afferents disappears due to desensitization simultaneously with the cessation of vomiting. This probably in part accounts for the broad-spectrum nature of the antiemetic efficacy of resiniferatoxin. Another factor contributing toward the broad antiemetic clinical potential of potent synthetic hybrid antiemetics is the concomitant stimulation of antiemetic CB_1 and TRPV1 receptors, which are distributed in a similar pattern in the neurons of the emetic nuclei of the dorsal vagal complex and are colocalized in the mNTS, in motor neurons of the DMNX, and in a few scattered neurons of the AP.[270] These findings further add to the broad-spectrum antiemetic nature of cannabinoids and vanilloids against both phases of chemotherapy-induced vomiting.[93,286,315] The antiemetic locus of CB_1 and TRPV1 receptor activity probably lies both in the vagal afferent/efferent neurons and NTS (see section 6.2). Indeed, recent multilabeling evidence indicates that CB_1 receptor immunoreactivity colocalizes not only with punctate terminal-like immunoreactivity for 5-HT or SP in the NTS but also in some puncta with both neurotransmitter antigens.[93] Activation of CB_1 receptors may also oppose the emetogenic effects of both 5-HT and SP at the level of the vagus and myenteric plexus (see sections 4.5 and 5.5). Like resiniferatoxin, anandamide can cause emesis in a nondose-dependent manner;[269] however, among the emetic agents tested, it provided protection against 2-AG and morphine-6-glucuronide but not cisplatin[266,269] (Darmani, unpublished observations).

7. Eicosanoids: Synthesis, Storage, Release, Degradation, And Receptors

The eicosanoids are a large family of 20-carbon molecules derived from the omega-3 and omega-6 essential fatty acids, eicosapentaenoic acid, arachidonic acid (AA), or gamma-linoleic acid. The eicosanoid family is further subdivided into smaller molecular families based on the initial biosynthetic enzyme(s) employed. Figure 8 describes the initial steps of the eicosanoid biosynthesis cascade, although it should be noted that, within each family of compounds shown as an "end point" in the figure, numerous related compounds are present. Indeed, the enormous variety of metabolites has slowed research on eicosanoid-mediated vomiting, in that no clear relationship between a particular family of metabolites and vomiting has yet been fully established. Rather, individual members of each family have been studied, usually in relation to nonvomiting related functions.

For all eicosanoids, biosynthesis begins when membrane phospholipids are catabolized via one or both of phospholipases A_2 and C to generate intracellular AA. Ingested (dietary) eicosapentaenoic acid and gamma-linoleic acid can also be absorbed or retrieved from cellular stores and are acted upon by the same cascades of enzymes that modify AA, thus forming parallel (and frequently competitive with AA-derived) metabolites. These essential fatty acids are then oxygenated via one of two primary mechanisms. Oxygen-

ation via lipoxygenases generates members of the leukotriene and hydroxyeicosatetraenoic acid families, whereas oxygenation via cyclooxygenases generates the prostanoid family. In addition to these enzymatic oxygenating steps, an alternate AA metabolic path involving phospholipases C and D is used to generate the endocannabinoids anandamide and 2-arachidonoyl glycerol (2-AG), which can be further acted upon by COX-2 to ultimately produce prostaglandin ethanolamides (prostamides) and prostaglandin glycerol esters. Yet another metabolic cascade, involving cytochrome P450 epoxygenase activity, generates epoxyeicosatetraenoic acid metabolites. Many eicosanoid metabolites are present or inducible in nearly all mammalian tissues. However, the AA-derived (omega-6) metabolites are enriched in immune cells, mast cells, and in smooth muscle cells, and are key mediators of smooth muscle tone and induction of inflammatory responses.[328−331] A further hallmark of eicosanoids in general is their relative instability, in that they are rapidly inactivated by dehydrogenase-mediated oxidation.[332−334] Thus, eicosanoids tend to be limited to paracrine or related local signaling actions. Further details on eicosanoid synthesis and receptor activities specific to particular eicosanoid families are described in the subsections below.

7.1. Leukotrienes

Generation of leukotrienes occurs when AA is converted by 5-lipoxygenase (5-LO) into 5-hydroxyeicosatetraenoic acid via an unstable peroxidated intermediary (5-HPETE). 5-LO then acts on 5-hydroxyeicosatetraenoic acid, producing leukotriene A_4 (LTA_4). This parent leukotriene can then be converted into LTB_4 or conjugated to the peptide glutathione to generate the cysteinyl leukotrienes. The parent cysteinyl leukotriene, LTC_4, can be stripped of a glutamic acid residue to form LTD_4, which can be stripped of its glycine residue to produce LTE_4. Additionally, LTC_4 can be converted via carboxypeptidase activity to LTF_4. The cysteinyl leukotrienes bind specifically, but with differing affinities, to two different receptor subtypes, the CysLT1 and CysLT2 receptors.[330] LTB_4, on the other hand, binds to either of two specific receptors dubbed BLT1 and BLT2.[335] Regardless of the receptor subtype, the listed leukotriene receptors are all members of the G-protein-coupled receptor superfamily, although it has been noted that the same receptor subtype can interact with different classes of G-proteins.[330]

7.2. Prostanoids

Prostanoids are generated by one of several isoforms of cyclooxygenase (COX), such that COX-1 is thought to be responsible for constitutive prostanoid synthesis, COX-2 is responsible for induced prostanoid synthesis (i.e., during inflammatory responses), and COX-3 is responsible for central nervous system signaling as well as inflammation.[331,336,337] The COX enzymes oxygenate the essential fatty acids via peroxide intermediates and generate a carbon ring structure in the middle of the fatty acid. The intermediate, prostaglandin G (PGG), is unstable and rapidly catabolyzes to PGH, the main parent molecule of the prostanoids. The derivatives are subdivided based on structure, such that prostaglandins have a 5-carbon ring, prostacyclins have a 5-carbon ring joined to an oxygenated ring or opened ring, and thromboxanes have a single 5-carbon ring with an included oxygen molecule. Numerous derivatives within each of these classes exist, and several receptor subtypes have been demonstrated that are specific for individual members of these classes, for example the thromboxane TXA_2 receptor, the $PGF_2\alpha$ receptor, and prostacyclin PGI_2 receptor. In addition, a series of four receptors for prostaglandin E_2 have been found. Prostanoid receptors are classified by the formula $XP_{\#}$, such that X represents the specific prostanoid ligand, and # represents the receptor subtype if present. Thus, TP receptors are specific for thromboxane prostanoids, and the EP_1-EP_4 receptors are subtypes of PGE_2-binding receptors. As was the case for the leukotrienes, the prostanoid receptors are G-protein coupled receptors.[329,338−341] However, certain other receptor types, in particular the nuclear peroxisome proliferator-activated receptor-γ, can also be activated by some prostanoids.[342−344]

7.3. Dorsal Vagal Complex

The amount of data collected on emesis-related eicosanoid activity in the dorsal vagal complex might lead one to conclude that eicosanoid-mediated vomiting is mediated elsewhere. What little information there is suggests that PGE_2 and possibly prostacyclin could modulate emetic behavior at the level of the dorsal vagal complex. EP_2, EP_3, and IP receptors are found throughout the NTS,[49,345,346] as is at least one isoform of COX.[347] In addition, the AP expresses EP_4 receptors.[346] The effect on the emetic reflex of stimulating these receptors has not been studied, although work related to autonomic control has consistently demonstrated that both PGE_2 and prostacyclin (PGI_2) reduce most cellular activity in the NTS,[348−351] although PGE_2 was also found to stimulate spontaneous, but not evoked, activity.[349] These data suggest a possible disinhibitory effect of prostanoids, in that Fos immunoreactivity has been found in the dorsal vagal complex following PGE_2[352] and LTC_4 administration (Chebolu, Wang, Ray, and Darmani, submitted for publication), although the exact subnuclei of the NTS expressing the protein have not been determined. Finally, several studies have revealed that cisplatin induced vomiting behavior and its related Fos immunoreactivity in the dorsal vagal complex can be significantly reduced through cannabinoid CB_1 receptor agonists,[93,277] suggesting eicosanoid-derived endocannabinoids could act as emetics/antiemetics through the dorsal vagal complex.

7.4. Vagal Afferents

Significantly more data exist that implicate eicosanoid modulation of nodose ganglionic vagal afferents as a key mechanism for eicosanoid-mediated vomiting. Emetogenic eicosanoid ligands bind to vagal afferent terminals via receptor subtypes that are typically (but not exclusively) coupled to G proteins that either stimulate cyclic AMP production[340,353,354] or calcium influx,[355,356] two conditions known to increase cellular activity and/or sensitivity. More specifically, these conditions are known to close a slow afterhyperpolarization current as a key step in enhancing cellular activity,[22,23,46,47,357−360] which is then expressed as increased vagus nerve neurotransmission[357,358,361] or potentiation of vagally mediated responses.[46] On the other hand, the prostaglandin PGE_2 was found to cause decreased vagal afferent activity on the NTS,[349] a seemingly contradictory effect in relation to emesis. However, the portion of the NTS targeted by these afferents may not have been the emesis-related mNTS, and different subtypes of EP receptors have been implicated that may distribute differently to emetic and

nonemetic vagal afferents. Indeed, the above mechanisms of eicosanoid-increased neural sensitivity have not been described in relation to vagal afferents confirmed to mediate emesis. Despite this, vagal afferent stimulation by 5-HT and/or SP is a verified critical substrate of emetic signaling (as detailed in their respective subsections), and 5-HT has been shown to produce the same neutralization of the vagal slow after-hyperpolarization current as the eicosanoids.[22,362]

Another potential mechanism of eicosanoid-mediated vomiting besides enhancing vagal afferent sensitivity and effector potency is directly related to 5-HT and/or tachykinin neurotransmission. In fact, 5-HT signaling appears necessary for several of the potentiating effects of the eicosanoids,[257,362−364] and vagal afferent neurons have the capacity to react to both 5-HT and prostanoids. Interestingly, 5-HT released from enterochromaffin cells not only acts on vagal afferents directly, by 5-HT_3 receptor activation, but also has the ability to release an SP-mediated stimulatory effect on vagal afferent neurons. However, this effect has only been demonstrated in neurons previously exposed to an immune challenge,[365] suggesting that inflammatory mediators (eicosanoids) are necessary for this synergy. SP and eicosanoid release also appear to be modulated in tandem, although this effect may be tissue-dependent. There is significant evidence that prostaglandins can stimulate SP release in sensory (nodose) neurons.[366,367] While most of the described eicosanoid effects are proemetic, the AA-related endocannabinoids can also inhibit 5-HT_3 receptor activity directly, seemingly through allosteric modulation,[325] providing a mechanism for vagally mediated cannabinergic antiemesis.

7.5. Enteric Nervous System

Several different eicosanoids have been found to act within the ENS, although there is very limited anatomical data regarding the presence of various eicosanoid receptor subtypes. The most studied are the PGE_2 receptors, of which EP_1, EP_2, and EP_3 receptors have been described as being localized in the ENS.[368] Most eicosanoid-related ENS data are pharmacological or electrophysiological in origin, but they suggest the presence of EP, DP, IP, and FP prostanoid receptors, as well as CysLT leukotriene receptors, in the ENS.[369−372] In addition, COX-2 has been localized to the ENS,[373,374] suggesting a paracrine or autocrine signaling mechanism is utilized for eicosanoid-mediated enteric activity. Despite the variety of potential receptors, eicosanoid application to the ENS appears to enhance neuronal excitability and/or spike discharge to produce two primary effects: (1) increased contractile responsiveness of intestinal smooth muscle either directly or through enhanced responsiveness to myenteric signals[368,372,375−379] and (2) increased neurosecretory activity in the mucosa.[369−372,380,381] These effects are mediated with second messengers similarly to the excitability-enhancing effect of eicosanoids on vagal afferents. This includes the above-described ability of eicosanoids to close a slow after-hyperpolarization current and increase neuronal sensitivity in the ENS itself.[372] In addition, cross-potentiation of 5-HT and SP signaling within the ENS can occur, either directly or through second messengers such as calcium and cAMP.[368,377,381] COX-2 activation in the ENS, probably via prostaglandin release, is also capable of enhancing neuronal excitability.[374] As both increased 5-HT and SP signaling in the ENS are heavily implicated in vomiting (detailed elsewhere in this review), especially cisplatin induced vomiting, this suggests that inhibition of prostanoid receptors and/or COX-2 suppresses vomiting via a combination of direct eicosanoid signaling inhibition, as well as indirect reductions in 5-HT and SP signaling. However, because nearly all of this work was performed using nonemetic animal models, the biochemical effects of ENS COX inhibition and prostanoid receptor antagonism are in need of further verification in emesis-capable animal tissue preparations.

7.6. Gastrointestinal Tissue

The eicosanoids can also act directly on cellular substrates in the gut wall and/or mucosa. In the non-neuronal tissue of the gastrointestinal tract, prostaglandin and leukotriene metabolites appear to be the most likely mediators of eicosanoid-mediated vomiting. Prostaglandin receptors have been identified in rabbit and rodent GI tissue samples, including EP_3 receptors in smooth muscle cells,[368] and EP_1, EP_4, and FP receptors in enterochromaffin cell isolates.[382] In fact, PGE_2 has been found to increase cAMP production in several gastrointestinal tract cell types.[354,368,381,382] Not surprisingly, PGE_2 signaling was found to cause a potent release of 5-HT from enterochromaffin cells,[364] an effect likely related to increased cAMP.[383] Cisplatin also induces vomiting in part through a massive release of GI-sourced 5-HT[18,21,384] (also detailed in section 9), while at the same time, higher levels of cAMP appear to increase cisplatin accumulation and cisplatin induced toxicity.[385] Thus, prostaglandin-mediated GI inflammation could be responsible for enhancing the potency of cisplatin induced vomiting, as well as being a source of emetic 5-HT release in its own right. However, it must be noted that these data have been produced primarily in nonemetic (e.g., rodent) animal models, or tissues culled from such animals, and therefore, more work validating these results in a vomiting animal model is necessary.

Non-neuronal gastrointestinal tract tissue may also use leukotriene metabolites as a proemetic paracrine messenger. For example, intoxication by *Staphylococcus* enterotoxin, a potently emetogenic toxin, causes significant increases in leukotriene production[386,387] and enteric mast cell degranulation[359] (release of AA metabolites, especially leukotrienes), which results in emesis.[387] Additionally, the cysteinyl leukotrienes LTC_4, LTD_4, and LTE_4 (but not the related derivative LTF_4) have been shown to be emetic in the least shrew, and the induced vomiting could be inhibited by the selective CysLT1 receptor-binding antagonist pranlukast (Chebolu, Wang, Ray, and Darmani, submitted for publication), suggesting a specific ligand−receptor interaction is engaged. Further complicating matters, there is a potential interaction between cisplatin and leukotrienes that may affect chemotherapy-induced vomiting as well as the effectiveness of the platinum-based chemotherapies themselves. The cysteinyl leukotriene LTC_4, by virtue of its glutathione moiety, can bind to the same solute-carrier membrane transporter that binds glutathione-conjugated platinum, a key cellular metabolite of cisplatin and the other platinum-based chemotherapies.[388,389] This competitive interaction has been exploited in experiments using LTC_4 treatment to successfully enhance the tumor-killing potency of cisplatin in glioma cells,[390] demonstrating that cisplatin resistance in cancer cells is at least partially mediated by active removal of the platinum−glutathione conjugate from the cell.[389,391] Essentially no work has been done studying this interaction with regard to the gastrointestinal tract, although some data

Table 1. Emetogenic Eicosanoids Grouped by Metabolic Family[a]

metabolite	evidence for emetogenic activity	references
Leukotrienes	release by mast cells is associated with vomiting	470
LTC_4	direct injection, animal	UD
LTD_4	receptor antagonists are antiemetic; CysLT2 receptors direct injection, animal	387, UD
LTE_4	receptor antagonists are antiemetic; CysLT2 receptors increased levels with enterotoxin challenge direct injection, animal	387, UD
LTB_4	increased levels with enterotoxin challenge	386
Prostaglandins	inhibition of synthesis reduces/blocks emesis	300, 398
PGD_2	direct injection, animal increased levels with cisplatin challenge	392, 406
PGE_2	direct injection, animal; EP_2/EP_4 receptors increased levels with enterotoxin or cisplatin challenge	257, 386, 392, 405, 407
$PGF_{2\alpha}$	direct injection, human and animal; FP receptors	407, 471
Prostacyclins		
PGI_2	no direct evidence; injection causes vagal excitation similarly to serotonergic agonist activity	22, 47, 358
Thromboxanes		
TXA_2	direct injection of mimetic agent, animal; TP receptors	363, 406
Endocannabinoids		
2-AG	direct injection, animal downstream metabolite may be active emetogen	300

[a] The metabolic family is italicized, and metabolites that are emetogenic, or demonstrate activity correlated with vomiting, are listed under the family name. Evidentiary data in which a particular metabolite is not specified (e.g., inhibition of biosynthetic enzymes) are listed in association with the metabolic family. Note that not all evidence given is direct, and correlative evidence is described as such where present. Where possible, the putative receptors involved are provided. "UD" represents unpublished data by the authors of this review.

have been acquired with regard to eicosanoids, cisplatin, and emesis. Indeed, one study[392] found that pretreatment with a glutathione prodrug prior to cisplatin treatment could abolish the rise in GI prostaglandin activity associated with cisplatin and vomiting in general. It is possible the high glutathione levels caused increased production of glutathione-conjugated, nonemetogenic prostaglandin metabolites (e.g., PGJ_2) and shifted the metabolic balance away from production of the pro-emetic prostaglandin metabolites (PGE_2 or $F_2\alpha$). Alternately, the high glutathione levels could have enhanced the metabolic conjugation of platinum and its removal from enterochromaffin cells via the plasma transporter, thus reducing gastrointestinal tract inflammation, 5-HT release, and cisplatin induced vomiting in turn.

Finally, the COX-2 inhibitor nabumetone and nonspecific COX inhibitor indomethacin can interact with 5-HT and SP signaling systems in the gut. Indomethacin has been shown to reduce SP signaling, in addition to prostaglandin synthesis, in rat stomach cells.[366] Nabumetone and indomethacin both were found to reduce 5-HT release induced by cisplatin from ileal enterochromaffin cells,[393] or in the stomach mucosa.[366] However, species differences in GI tissue transmitter/eicosanoid/receptor content can once again be problematic. For example, indomethacin has been shown to be antiemetic,[300,327] proemetic,[394] and completely ineffective[395] with regard to chemotherapy-induced vomiting, apparently depending on the species tested. Indomethacin has not been used solely as an antiemetic in human clinical cases, but in trials for dysmenorrhea, it was moderately effective in stopping the associated vomiting.[396]

7.7. Eicosanoids and Emesis

Clinically, the synthetic corticosteroid dexamethasone is used either as a primary antiemetic treatment or, more frequently, in conjunction with other classical antiemetics (e.g., $5\text{-}HT_3$ antagonists). Dexamethasone has multiple activities in vivo, including glucocorticoid receptor binding, inhibition of prostanoid synthesis, and membrane stabilization.[395] While the exact mechanism of dexamethasone mediated antiemesis is unknown, several sources suggest that the major component of its antiemetic actions may not be inhibition of prostanoid synthesis. For example, in pigeons and in some instances in ferrets, the nonselective COX inhibitor indomethacin failed to inhibit cisplatin induced vomiting under the same treatment conditions in which dexamethasone was successful,[395] whereas in decerebrate cats pretreatment with a GR receptor antagonist successfully antagonized the antiemetic effect of dexamethasone on xylazine-induced vomiting.[397] However, as is frequently the case, species differences, the use of different emetogens, and the emetic doses employed cloud the results, but inhibition of prostaglandin synthesis by COX-specific inhibitors (including indomethacin), especially the inducible (COX-2) isoform, has often been found to be antiemetic.[300,394,398] Indeed, as shown in Table 1, some downstream products of COX enzymes such as PGE_2 and $PGF_{2\alpha}$ are emetogenic in several species including humans,[399] piglets,[400] and least shrews.[300] In addition, the rise in plasma concentrations of the cited prostaglandins, some leukotienes, or 5-hydroxyeicosatetraenoic acid is associated with vomiting under some conditions including pregnancy in humans[401] and *S. aureus* enterotoxin B exposure in monkeys.[386]

Synthesis of leukotrienes by 5-LO, already known to be a key factor in asthma,[355,402] may also modulate inflammation-mediated emetic behavior, although supporting data are very limited. Although not specifically related to emesis, blockade of leukotriene biosynthesis has been found to reduce the potency of tachykinin-related neurotransmission,[402,403] an effect potentially antiemetic depending on the affected targets. The antiemetic potential of leukotriene mediated attenuation of tachykinin transmission is yet to be verified, however, since the tissue preparations used were from nonemetic animals. Further muddying the picture, blockade of leukotriene biosynthesis with the specific inhibitor MK-886 failed to affect cisplatin induced emesis[394] in the ferret emetic model, but as discussed earlier several leukotrienes

are potent emetogens in the least shrew. More research is necessary to determine whether inhibition of leukotriene synthesis is ineffective for all forms of emetic induction, or whether this lack of effect is specific to chemotherapy-induced vomiting or to species differences.

Although overall research of eicosanoid-mediated vomiting has lagged relative to other emetic mediators (e.g., 5-HT) and there are significant gaps in our understanding of the phenomenon, some interesting data have been obtained that highlight the wide reach of the AA signaling cascade. For example, endocannabinoids such as anandamide and 2-AG are antiemetic in the ferret at low doses,[270,273] but in the least shrew, evidence suggests 2-AG can be converted back into the proemetic metabolites, arachidonic acid and/or prostamides.[51,269,404] Also, a variety of AA metabolites that span the different eicosanoid families are proemetic, including thromboxane TXA_2, prostaglandins $PGF_2\alpha$ and PGE_2, and leukotrienes LTC_4 and LTD_4 (see Table 1). In fact, in the least shrew, systemic administration of LTC_4 not only caused vomiting (see above) it also resulted in Fos expression in the emetic nuclei of the dorsal vagal complex and in the ENS (Chebolu and Darmani, submitted for publication). A short list of known emetogenic eicosanoids has been presented in Table 1 (and structures presented in Figure 8) to demonstrate both the breadth of eicosanoid members that can mediate eicosanoid-mediated vomiting and the few metabolic products that have been emetogenically characterized relative to the number of known metabolites.

While the currently accepted dogma of chemotherapy-induced vomiting as described in section 9.1 invokes the primacy of one anatomical compartment (i.e., peripheral or central compartments) at a time, and sequential activation (e.g., peripheral 5-HT release in the acute phase vs central SP activity in the delayed phase) rather than overlapping activation, eicosanoid mediation of emesis suggests peripheral and central activation could be engaged together. Eicosanoids have a strong effect on gastrointestinal tract motility via paracrine signaling,[377,405] which suggests a peripheral mechanism is in play. Furthermore, because eicosanoids are degraded so rapidly, it is unlikely that a sequential effect induced by increased eicosanoid circulation would occur. Thus, Fos expression that is induced in both enteric and central neurons following LTC_4 injection-related vomiting (Chebolu, Wang, Ray, and Darmani, submitted for publication) would be more likely related to activation of both enteric and central emetic nuclei at approximately the same time. Further support of combinations of central and peripheral activity comes from the data described in detail above, in which mechanisms of eicosanoid-mediated vomiting have been described for studies using both intraperitoneal[377,406] and central[363,397] administration. In fact, the latter cited studies are especially telling, in that administration of a prostaglandin receptor agonist directly into the fourth ventricle potently induced emesis that was refractory to abdominal vagotomy, and central administration of dexamethasone into the NTS also prevented systemically administered, agonist-induced vomiting. Thus, a gestalt of the data strongly implicates both peripherally and centrally mediated effects of eicosanoids in the emetic reflex.

Although the idea that so many eicosanoids are potentially emetogenic may appear daunting, especially to clinicians attempting to find better antiemetic drugs, one can take comfort by the research, which suggests there are only a few common mechanisms involved in eicosanoid-mediated vomiting. Rather, the potential of interspecies differences, and of different emetogens utilizing mechanisms insensitive to eicosanoid modulation, are the greater problems currently facing antiemetic agent development. For example, Kan and colleagues noted differences between musk shrews and ferrets in the ability of various prostanoid receptor agonists to induce vomiting.[406,407] Also, while cysteinyl leukotriene receptors are able to mediate the increased vagal excitability described as a putative mechanism of eicosanoid-mediated vomiting (described below), vomiting induced by mountain sickness (and possibly other emetogenic conditions) is not affected by the specific CysLT1 receptor antagonist montelukast.[408] However, this does not rule out a CysLT2 receptor-mediated mechanism. Other evidence in the guinea pig suggests a third CysLT receptor subtype may exist that mediates colonic activity.[372]

As mentioned, the AA signaling cascade induces a broad range of cellular functions. However, several of its key functions stand out as putative mechanisms of eicosanoid-mediated vomiting. For example, studies using different AA metabolites have demonstrated a consistent ability of NK_1 receptor antagonists to reduce or block emesis induced by direct eicosanoid injection.[257,363] This interaction also provides a mechanism for linking the sensing of noxious stimuli (by tachykinins), or the inflammation caused by the noxious stimulus, to a suitable protective behavioral output (emesis). Thus, despite the wide reach of AA metabolites, several common themes—5-HT or tachykinin-related neurotransmission, G-protein coupling, and metabolite-specific receptor-binding—are found across the eicosanoid families, essentially funneling these diverse compounds into several major mechanistic pathways for producing eicosanoid-mediated vomiting.

8. Interactions among Emetic Neurotransmitters

Thus far, we have considered the various neurotransmitters and intercellular messengers as individual systems that act and are acted upon independently of each other. However, there are also significant interactions among these systems, which can occur through several mechanisms including colocalization of transmitters or receptors to the same cells, presynaptic heteroreceptor binding, or traditional neurotransmitter-based interconnections between neurons. In addition, extracellular release of paracrine signaling molecules such as prostanoids or endocannabinoids can modify the release of other neural/endocrine signals.

For example, one of the most well-defined interactions occurs between serotonergic and dopaminergic systems in the brain. Serotonergic and dopaminergic neurons demonstrate convergent input to several brainstem nuclei, including the locus coeruleus and dorsal vagal complex.[409,410] In addition, dopaminergic and serotonergic nuclei frequently exchange reciprocal innervation, with several different serotonergic receptors noted on the dopaminergic neurons, including $5\text{-}HT_{2A/2C}$ and $5\text{-}HT_3$ subtypes.[411−413] The importance of $5\text{-}HT_3$ receptors in chemotherapy-induced vomiting suggests that an interaction between DA outflow and 5-HT acting at these receptors may modulate emesis as it does limbic activity.[411,413] Although increases in 5-HT and DA are potential mechanisms of chemotherapy-induced vomiting, the direct interactions between DA and 5-HT appear to be inhibitory.[324,414] This also holds true for their interactions in the brainstem.[415] Indirectly though, 5-HT can either increase or decrease DA outflow, depending on brain region, via

serotonergic heteroreceptors on GABAergic interneurons within dopaminergic nuclei.[324] Thus, either convergent inputs to the dorsal vagal complex or indirect modulation relayed through interneurons could provide a mechanism through which 5-HT and DA might interact to induce or enhance emetic responses.

Interactions between the tachykininergic system and both 5-HT and DA are also frequent. SP innervates the serotonergic dorsal raphe nucleus, where it both enhances interneuron activity and directly reduces (via NK_1 receptors) 5-HT neuron activity.[256,416] SP not only innervates the ventral medullary raphe nuclei but also colocalizes to a subset of serotonergic raphe neurons that project to the NTS.[417] The relevance to chemotherapy-induced vomiting of this particular projection has not been studied. However, a tachykinin-5-HT interaction highly relevant to chemotherapy-induced vomiting occurs at the level of the vagal afferent terminals, which contain both NK_1 tachykininergic and 5-HT_3 serotonergic receptors. Antagonists of NK_1 receptors can block vagal afferent activation (a hallmark of chemotherapy-induced vomiting) induced by 5-HT_3 receptor stimulation, and vice versa, in the ferret.[38] Immunohistochemical labeling in the nucleus of the solitary tract demonstrated punctate, terminal-like labeling for 5-HT and SP, both separately and colocalized,[93] suggesting that the NK_1 and 5-HT_3 receptors could be activated simultaneously or individually during chemotherapy-induced vomiting. In the gastrointestinal tract, SP-immunoreactive fibers project from the submucosal nerve plexus to close apposition with NK_1 receptor- and 5-HT-containing enterochromaffin cells[418] (Ray, Chebolu, and Darmani, unpublished data), and rare colocalization of 5-HT- and SP-immunoreactive neurons have also been noted.[419] SP is not limited to 5-HT in its interactions, however, in that it interacts with dopaminergic systems as well. In general, SP acts to inhibit dopaminergic activity and vice versa,[420,421] although peripheral administration of SP paradoxically increased striatal DA release.[237] Resolving the question of chemotherapy-induced vomiting-specific interactions between SP and DA will require further study.

Finally, the above-mentioned neurotransmitter systems can interact with paracrine signals, including cannabinergic and inflammatory signals. CB_1 cannabinoid receptors are found on many terminals throughout the dorsal vagal complex (especially the NTS) as well as throughout the enteric nervous system and are sometimes colocalized to terminal- or fiber-like labeling for SP and 5-HT.[93,422] Furthermore, CB_1 receptors are found on neurochemically identified serotonergic, tachykininergic, and dopaminergic neurons[423] (Ray, Chebolu and Darmani, unpublished data). Endocannabinoids are released from dendrites and act presynaptically, where stimulation of CB_1 receptors inhibits neurotransmitter release. Most importantly for chemotherapy-induced vomiting, by reducing serotonergic, dopaminergic, and possibly tachykininergic neurotransmission in the dorsal vagal complex and/or gastrointestinal tract, cannabinoids generate an antiemetic effect.[51,273,306,316] Despite the limited anatomical evidence, clear pharmacological evidence for chemotherapy-induced vomiting-relevant cannabinoid interactions exists. In the least shrew, for example, cannabinoid pretreatment inhibits vomiting induced by dopaminergic,[111,316] serotonergic,[168] or tachykininergic[318] agents. Indeed, CB_1 receptor antagonist (SR141716A) administration to least shrews causes not only vomiting but also release of 5-HT and DA.[306] In addition, cannabinoid pretreatment enhances the 5-HT_3 receptor-mediated attenuation of cisplatin induced vomiting.[323] There is also evidence for direct, allosteric modulation of the 5-HT_3 serotonergic receptor by the endocannabinoid anandamide, providing evidence for a potential mechanism for the above-described serotonergic/cannabinoid interaction.[168,325,424] Interestingly, in the brainstem, 5-HT can induce endocannabinoid release via 5-HT_2 receptor activation, which suppresses activity at glutamatergic synapses,[425] while in the basal ganglia dopaminergic activation of D_2-like receptors can likewise induce endocannabinoid production.[426,427] Thus, these interactions essentially act as local negative feedback loops at particular serotonergic or dopaminergic synapses. The interaction of tachykininergic and endocannabinoids is only just being delineated, although several studies have noted interactions between CB_1 and NK_1 receptor-mediated behaviors,[252] which suggest cannabinoids, as they do with other neurotransmitter systems, act to inhibit SP neurotransmission.[428] Taken together, these findings provide good evidence that the interaction of cannabinoids with serotonergic, dopaminergic, and/or tachykininergic terminals is necessary for the antiemetic behavioral effects of phytocannabinoids and synthetic CB_1 receptor agonists. Eicosanoid (arachidonic acid-derived) paracrine signals such as prostaglandins can also interact with the various neurotransmitter systems. This interaction appears to take place at least at the level of signal transduction, where prostanoids have been shown to increase cAMP levels in enterochromaffin cells,[364] inducing secretion of 5-HT from the intestinal mucosa (a key step in chemotherapy-induced vomiting). Indeed, inflammatory responses mediated by eicosanoids and the secretion of 5-HT appear intimately connected, such that inflammation enhances release of 5-HT by the mucosa, and 5-HT activation of various receptor subtypes induces eicosanoid and cAMP production.[374,380] Very little is known about eicosanoid interaction with dopaminergic signaling in regards to chemotherapy-induced vomiting, although ileal enterochromaffin cells express dopaminergic receptors,[382] so the possibility exists that eicosanoids interact with DA signaling via second messengers in the same way they interact with 5-HT signaling. With regard to SP, regional and cell-specific effects of eicosanoids have been found that suggest leukotrienes can suppress tachykininergic signaling postsynaptically, or possibly through interaction via astrocyte activity.[403,429] As mentioned, enterochromaffin cells also express NK_1 receptors, and eicosanoids could potentially interact with tachykininergic signaling as described above for serotonergic and dopaminergic signaling in the gut.

9. Chemotherapy-Induced Nausea and Vomiting

Although in the past decade targeted molecular therapies are increasingly being used in cancer treatment, classical chemotherapy still remains the mainstay of cancer treatment for most patients with advanced malignant disease that is incurable by local surgery or radiotherapy. The clinical utility of chemotherapeutics can become limited by their adverse effects, with nausea and vomiting being the most severe of these, forcing patients to postpone or refuse treatment. Chemotherapeutic agents can be classified into four risk groups in terms of their emetogenic/nausea potential in patients: high (>90% of patients, e.g., cisplatin), moderate (30−90% of patients, e.g., cyclophosphamide), low (10−30% of patients, e.g., methotrexate) and minimal (<10% of patients, e.g., bleomycin).[430,431] This section will re-examine the established neurotransmitter basis of chemotherapy-

induced vomiting since it is becoming increasingly evident that the time-honored dogma requires updating. Since detailed evidence for each of the well-recognized neurotransmitters involved in emesis per se has already been presented in terms of their central and peripheral mechanisms in the context of brainstem/gastrointestinal tract circuits, this section will explore the existing evidence as it relates to the mechanisms concerning how, where, and when cisplatin acts in the emetic loci to cause both the immediate and delayed phases of chemotherapy-induced vomiting in humans and vomit-competent animals. Mechanistic focus will be mainly based upon cisplatin since this agent is best investigated and is one of the most efficacious emetogens, and depending upon the emesis model considered, chemotherapy-induced vomiting duration can vary from 2 to 7 days. Finally, the clinical usefulness relative to basic success in animal models for different classes of antiemetics against chemotherapy-induced vomiting will be briefly discussed.

9.1. Critical Analysis of the Neurotransmitter Basis of Acute and Delayed Cisplatin-Induced Emesis

9.1.1. Currently Accepted General Characteristics of Chemotherapy-Induced Vomiting Across Emesis Competent Species

Chemotherapy-induced vomiting involves both central and peripheral mechanisms. However, the mechanistic details of implicated emetic neurotransmitters and their interactions at each site have not yet been fully established.[9,210,258,432] The previously discussed medullary dorsal vagal complex nuclei are involved in the central mediation of chemotherapy-induced vomiting, while the vagus, myenteric nerves, and intestinal mucosa comprise some of the peripheral components. Together these form the brainstem−gut circuits that control production of emesis (see also Figure 1). Although these circuits are poorly understood, it appears that the previously discussed emetic stimuli, DA, 5-HT, SP, and prostaglandins all contribute to its genesis.[18,73,210,258] The advent of platinum-based antineoplastic therapies, beginning with cis-diamminedichloroplatinum(II), or cisplatin, and continuing with its next-generation congeners such as oxaliplatin and carboplatin, has been a boon in the fight against many types of tumors. The potency of the "platins" stems from their ability to undergo nucleophilic reactions with G-C rich nucleic acid segments, forming cross-links and adducts that block cell division and/or induce apoptosis (programmed cell death). Although potent against many tumor types, a major drawback to the platins is their relatively high toxicity toward noncancerous cells, especially in the gastrointestinal tract, kidneys, peripheral nerves, and ears. Cisplatin-induced formation of reactive oxygen species contributes to its cytotoxicity[433] and accumulation of such free radicals in the enterochromaffin cells is thought to cause local exocytotic release of emetogens including 5-HT necessary for the induction of emesis.[150]

Cisplatin exposure produces vomiting biphasically in both humans[7] and other emetic species.[170,258,259,434,435] In patients, the acute (immediate) emetic phase comprises episodes occurring within 24 h of cisplatin exposure and the delayed phase between days 2−7 postinfusion. A close inspection of the published studies in animal models of chemotherapy-induced vomiting suggests the details of temporal development of cisplatin induced emetic behaviors are somewhat variable and are probably dependent upon (1) the dose used, (2) the route of administration employed, (3) the presentation of attained emetic parameters either as a single behavior or a combination of behaviors, and (4) the possible species differences in cisplatin action and disposition. Indeed, as with humans,[7] cisplatin was infused in piglets (5.5 mg/kg at 0.374 mg/min), but the immediate and delayed peaks occurred at 2 and 22 h respectively, and emetic events lasted up to 58 h.[436] A similar profile occurred in the cat as cisplatin (5 mg/kg, i.v.) induced maximum emetic behaviors between the third and fourth hours for the immediate phase succeeded by a quiescent period up to 22 h and then followed by a series of delayed episodes between 22−38 h.[437] A larger cisplatin dose (7.5 mg/kg) induced both phases in the cat quicker but toxicity restricted the full observation time. Likewise, in pigeons, cisplatin (4 mg/kg i.v.) caused immediate and delayed peak behaviors, respectively, at 2−4 and 16−22 h postcisplatin treatment with lower frequencies being present throughout the 48 h observation period during which some pigeons died of toxicity.[170] In the ferret, intraperitoneally administered cisplatin (5 mg/kg) produced the corresponding immediate and delayed peaks at 3−4 and 52−56 h, respectively, while a 10 mg/kg dose caused the emetic effects earlier, but toxicity limited full observation.[434] In the least shrew, a 10 mg/kg intraperitoneal injection of cisplatin produced both phases of chemotherapy-induced vomiting with corresponding peak mean vomit frequencies occurring at 2−3 and 33 h post-treatment. At 5 mg/kg it failed to cause significant emesis in either phase, while its 20 mg/kg dose induced both phases earlier but toxicity restricted the 47 h observation.[258] The house musk shrew (*S. murinus*) appears not to be very sensitive to the emetic effects of cisplatin. Indeed, only 80% of the animals vomited in response to the largest tested dose of cisplatin (40 mg/kg), and toxicity caused fatality in a significant number of these animals and thus limited full observation.[435] Therefore, comparisons of antiemetic efficacy within the same or different classes of antiemetics across varied species must consider both the duration of antiemetic half-lives and the emetic dosage of cisplatin employed. In addition, biochemical analysis of subsequent changes in neurotransmitter turnover in the brain, gut, plasma, or urine should be made appropriately.

The current antiemetic therapy dogma is based upon the premise that, during acute vomiting, cisplatin induces 5-HT release from enterochromaffin cells, which stimulates local 5-HT_3 receptors on gastrointestinal vagal afferents to initiate the vomiting reflex.[150] The delayed phase emesis is thought to be due to activation of brainstem tachykininergic NK_1 receptors subsequent to the release of SP in the dorsal vagal complex.[210] However, by itself, this hypothesis is too simplistic, as it is mainly focused on one neurotransmitter in isolation per emetic phase via a well-established mechanism in either the gastrointestinal tract or brainstem, respectively, and excludes interactions not only between emetic neurotransmitters at each peripheral and CNS emetic locus, but also between brain−gut emetic circuits. In addition, many contradictory findings are still ignored. Indeed, the discussed dogma was based on initial acute neurotransmitter release studies in which changes in 5-HT/5-HIAA turnover were determined either in animal gut[393,438] or in human plasma/urine samples,[439−441] as well as pharmacological, electrophysiological, and lesioning studies.[41] The ex vivo animal experiments were generally restricted to less than 6 h of

cisplatin exposure, while human studies were often confined to less than the first day of chemotherapy exposure, or 5-HIAA samples were analyzed at relatively long intervals during the delayed phase which could have masked the observed changes. Indeed, in two of the human studies,[439,440] smaller 5-HIAA peaks postday 1 exposure were observed but ignored to fit the accepted dogma. However, more recent detailed studies seem to challenge the initial work upon which these concepts are based.

9.1.2. Movement toward a Revision

A relatively recent clinical study clearly shows a more complex, differential, and overlapping involvement of increased 5-HT and SP turnover in human serum/urine during both phases of chemotherapy-induced vomiting.[8] The latter findings are further supported by a new animal study in which large increases in 5-HT turnover (as well as DA and SP turnover to be discussed later) in the least shrew brainstem and jejunal tissues were shown to occur during both peak immediate and delayed phases of cisplatin induced emesis.[258] Previous evidence for increased intestinal 5-HT turnover during delayed emesis did exist, but again was often ignored to correspond with the established doctrine.[442,443] Until recently, another contributory factor for the delay in modernization of the neurotransmitter basis of chemotherapy-induced vomiting has been lack of multitransmitter turnover studies run in parallel in both the brain and gut following chemotherapy exposure. Indeed, the relevant cited studies were published in separate manuscripts by different investigators who focused either on early or delayed cisplatin exposure, or either in the brainstem or small intestine. In terms of a peripherally mediated vagal mechanism, a large body of published neurochemical, electrophysiological, and behavioral evidence exists in support of a major peripheral role for gastrointestinal 5-HT in the mediation of the acute phase of cisplatin induced emesis in accordance with the current tenets (see section 4.5 and ref 150). These findings along with the newly published neurotransmitter turnover studies in both phases of chemotherapy-induced vomiting provide a plausible partial explanation as to why 5-HT_3 receptor antagonists are firstline clinical antiemetics for the acute phase but are only able to improve the efficacy of other classes of antiemetics and not by themselves block emesis during the delayed phase.[9,444]

A second major problem with current chemotherapy-induced vomiting neurotransmitter dogma is ignorance of significant published support for a cisplatin induced increase in brainstem 5-HT turnover in mediating one or both phases of emesis. Indeed, intraperitoneal administration of cisplatin not only increases indices of 5-HT function in both the ferret and least shrew brainstem during the acute phase,[258,442,445] it also increases 5-HT turnover in the latter emetic locus during the delayed phase.[258,443,446] A more direct role for brainstem 5-HT in cisplatin induced vomiting is again suggested by reports that central administration of peripherally ineffective doses of either (1) 5-HT can cause acute emesis in ferrets and marmoset monkeys[120,167] or (2) cisplatin can induce acute or delayed emesis in pigeons and cats.[169,170,395] Thus, it seems reasonable to assume that cisplatin may release 5-HT locally within the dorsal vagal complex to induce vomiting, since its peripheral administration increases not only the brainstem level of cisplatin itself[395] but also the tissue concentration of 5-HT in this region.[258,442,443,445,446] Moreover, while intraperitoneal injection of 5-HT in both least and house musk shrews causes emesis, the foremost animal model on which the chemotherapy-induced vomiting neurotransmitter dogma is mainly based, the ferret, does not vomit in response to peripherally injected 5-HT.[91,165,447] Other evidence in support of a direct CNS emetic component comes from reports that central but not systemic injection of quaternary forms of 5-HT_3 receptor antagonists (which are unable to pass the blood−brain barrier) prevent emesis produced via systemically administered cisplatin in dogs,[171] despite existing contradictory evidence in the ferret.[150,448] Since cisplatin causes a massive release of 5-HT in the gastrointestinal tract, which contains 95% of total 5-HT content in the body (see section 4), it is important to know whether or not peripherally released 5-HT would contribute to vomiting by penetrating the blood−brain barrier. However, 5-HT is a highly charged molecule at physiological pH, and it is expected not to pass the blood−brain barrier at least at physiological concentrations, since the brain microvessel endothelial cells form a continuous layer of cells and extracellular matrix, tight junctions, and reduced pinocytosis. Nevertheless, 5-HT may gain entrance through the AP or other circumventricular organs in the CNS, since large doses of peripherally administered 5-HT can induce centrally mediated behaviors such as wet dog shakes and scratchings whose loci of initiation in rodents are in deeper regions of the brain.[449] Indeed, significant amounts of peripherally administered ^{14}C-labeled 5-HT were detected in the rat brain 4 and 8 h following its systemic administration.[450] In addition, under conditions of stress, 5-HT is capable of opening the blood−brain barrier via 5-HT_2 receptors from the luminal side by a Ca^{2+}-dependent mechanism.[451] Likewise, other emetogenic inflammatory mediators that are coreleased by cisplatin such as AA and related metabolites increase the permeability of the blood−brain barrier. Thus, there is the possibility that, during the full time course of chemotherapy-induced vomiting, peripheral 5-HT and/or other inflammatory mediators could enter the CNS in general and the brainstem in particular. However, under nomal conditions, intraperitoneally or subcutaneously administered emetic doses of 5-HT up to 5 mg/kg did not produce any other CNS-mediated behavior in the least shrew, while a similar dose of its brain-penetrating 5-$HT_{2/3}$ selective analogue, 2-methylserotonin, simultaneously caused head-twitches, scratches, and vomiting in this emesis competent model.[168] Thus, the differential ability of 5-HT to induce head shakes in rats but not its equivalent behavior in least shrews may be due to species differences, and indeed significant evidence suggest that 5-HT may enter the brain of some species but not others.[452]

At the time of introduction of NK_1-receptor antagonists for basic research, the direct role of SP in vomiting and the involvement of NK_1 receptors in emesis were not established. However, since the 1980s, numerous indirect evidence such as histochemical, electrophysiological, and pharmacological antagonist studies have accumulated to implicate the involvement of SP in emesis via activation of its NK_1 receptor in the dorsal vagal complex emetic nuclei (see section 5). Thus, no direct supporting evidence in terms of SP release in the brainstem following cisplatin treatment or induction of emesis by selective NK_1 receptor agonists was available (see section 5.1 and ref 210). Nevertheless, the chemotherapy-induced vomiting neurotransmitter dogma advocates the involvement of specific release of SP in the medial subnucleus of the NTS and subsequent activation of emetic NK_1 receptors and production of vomiting during the delayed

phase of cisplatin induced emesis. Indeed, based upon pharmacological studies in vomit-competent animals, NK_1 receptor antagonists are used clinically in conjunction with standard antiemetic therapy during delayed phase chemotherapy-induced vomiting.[9] Recently it has been shown that not only does intraperitoneal injection of brain-permeable selective NK_1 receptor agonists induce emesis in the least shrew,[92] but also cisplatin increases SP release in the brainstem of this vomit-competent species.[258] In fact, we have established that both SP and its brain-penetrating selective NK_1 receptor agonist, GR73632, but not its brain-impermeable selective NK_1 agonists, induce vomiting via activation of NK_1- (but not NK_2- or NK_3-) receptors located in the NTS and the DMNX. Furthermore, GR73632 significantly increased emesis-related Fos-immunoreactivity in the shrew NTS and DMNX but not in the AP, indicating selective activation of dorsal vagal complex emetic nuclei. On the other hand, cisplatin causes broader Fos activation in all of these dorsal vagal complex nuclei during the peak immediate phase, whereas in the peak delayed phase Fos-immunoreactivity was not induced at all in the AP, and lower levels of Fos induction were seen in the remaining dorsal vagal complex nuclei when compared to the acute phase.[93] Unlike SP, peripheral injection of GR73632 also induced a centrally mediated scratching behavior that is thought to be mediated in deeper structures of the brain to which SP cannot gain access.[92] In a subsequent study, we confirmed that cisplatin causes tremendous increases (1396% and 956%, respectively) in brainstem SP tissue levels both during peak immediate and delayed phases of chemotherapy-induced vomiting, with the increase being maximal in the initial phase. Although this new observation argues against the SP basis of established chemotherapy-induced vomiting neurotransmitter dogma, it does explain why 5-HT_3 receptor antagonists are unable to completely prevent chemotherapy-induced vomiting in cancer patients during the immediate phase, even though combinations of both NK_1 and 5-HT_3 classes of antiemetics potentiate each others' efficacy during both chemotherapy-induced vomiting phases.[9,444] Thus, it appears reasonable to conclude that local increases in brainstem SP turnover are probably involved in both chemotherapy-induced vomiting phases, since i.c.v. injection of peripherally ineffective doses of cisplatin produces both emetic phases in an NK_1 antagonist sensitive manner in pigeons.[170] An important remaining issue is whether locally released SP in the brainstem is the only source of this emetic peptide during chemotherapy-induced vomiting, or whether peripherally released SP can also take part by entering the brainstem and thus contributing to cisplatin induced vomiting. As discussed earlier in section 5.5, there is substantial evidence that SP is unlikely to pass the blood−brain barrier under physiological conditions in sufficient quantity to cause vomiting, but when massive amounts of SP are released in the gastrointestinal tract during cisplatin exposure (or following peripheral injection of large doses of SP), the peptide could enter rapidly into the brainstem via a specific carrier-mediated transport mechanism in the AP and/or NTS.[240,241] In fact, following intraperitoneal injection of an emetic dose of SP in the least shrew, the brainstem, but not frontal cortex, tissue concentration of the peptide rapidly increased, a finding that is exactly mirrored in the rats following its intracarotid injection.[249] These findings correspond well with SP-induced emesis, in that the onset of vomiting was within 1−2 min of injection, and the remaining episodes occurred mainly within 5 min of administration.[92] A similar emetic profile is seen in the dog following intravenous injection of the peptide.[223] Thus, relative to the discussed possibility of contribution of peripheral 5-HT causing direct dorsal vagal complex-initiated emesis in the CNS via entrance into the brainstem (see section 5.5), there appears more certainty in the probability of peripherally released SP contributing toward direct central initiation of chemotherapy-induced emesis.

Indeed, a similar pattern but relatively more limited increases (333% and 226.5%, respectively) in least shrew jejunal SP tissue concentrations has been observed during both peak phases of cisplatin induced emesis.[258] A recent clinical study supports the latter findings, since changes in the plasma SP concentration of patients receiving high dose cisplatin had a similar profile of increases in both acute and delayed phase chemotherapy-induced vomiting.[8] The observed changes in the least shrew study seem to be region specific, since the shrew frontal cortex SP concentration during the delayed phase was decreased, while duodenal concentrations were unaffected in either phase. The changes in intestinal SP turnover in the periphery may affect induction of vomiting since the gastrointestinal tract can be another potential anatomical substrate for emesis. Indeed, both SP and NK_1 receptors are present on vagal afferents, in the ENS, and in intestinal tissue, which can directly or indirectly stimulate intestinal motility (see section 5). In fact, though Fos-immunoreactivity was frequently noted in the shrew ENS independent of emesis, in shrews that vomited in reponse to intraperitoneal injection of GR73632, a modest but significant increase in Fos-immunoreactivity in the ENS was found.[92] Furthermore, specific ablation of NK_1 receptors from a small region of least shrew small intestine by SSP-saporin causes profound quantitative and qualitative changes in the ability of GR73632 to induce emesis.[92] Indeed, in addition to a reduction in the number of shrews vomiting in response to varying doses of GR73632, the NK_1 receptor-ablated shrews also exhibited significantly smaller mean frequencies of vomits. Interestingly, while the largest tested dose (5 mg/kg) still induced emesis in all ablated shrews, these animals were unable to execute each vomit normally. Rather, the rhythmic retching movements with corresponding mouth openings, which normally required 2−4 s to expel the vomit in naive shrews, required 15−30 s for the completion of each ejection, possibly because these animals were unable to generate a significant retroperistaltic intestinal movement to expel the vomit. Despite the demonstrable peripheral lesion, i.p. SSP-saporin neither eliminated brain SP-immunoreactivity nor completely eliminated GR73632-induced emesis. Furthermore, the centrally mediated GR73632-induced scratching behavior in ablated shrews was similar in number to those in saline-injected control shrews. Thus, these findings, as well as those in section 5, provide solid evidence for a mixed central/peripheral activity for SP in both phases of chemotherapy-induced vomiting and further suggest that activation of gastrointestinal tract NK_1 receptors is not required for the initiation of the vomiting process but only for rapid execution of vomit expulsion. Combined with the ability of SP to generate retroperistalsis,[235] and to relax the lower esophageal sphincter (an event occurring in emesis) via NK_1 receptors,[236] these findings provide compelling evidence to suggest involvement of peripheral NK_1 receptors in vomiting. The above evidence again argues against the current simple notion of chemotherapy-induced vomiting neurotransmitter dogma,

which has implicated a CNS-only role in the delayed phase of chemotherapy-induced vomiting. The currently proposed hypothesis fits well with clinical observations that inclusion of NK_1 receptor antagonists in antiemetic cocktails improves their overall efficacy during both phases of chemotherapy-induced vomiting.[9,444]

Another deficiency of the current chemotherapy-induced vomiting neurotransmitter dogma and related published reviews is the sparse discussion of the basic science of the dopaminergic aspect of chemotherapy-induced vomiting. The clinical role of dopamine D_2 receptors in chemotherapy-induced vomiting and utilization of its corresponding antagonists are generally thought to be of historical interest, though these agents were the mainstay of antiemetic therapy prior to the advent of "setron" 5-HT_3 antagonists.[73,259] This is not very surprising, since until recently little basic or clinical work on the effect of cisplatin on DA turnover in any of the discussed peripheral or central emetic loci has been published. Indeed, exposure to cisplatin was shown to increase DA function in either a PC12 cell line or in the plasma of cancer patients receiving chemotherapy.[453,454] However, a recent study in the least shrew brainstem and jejunum clearly showed increased DA turnover associated with peak immediate and delayed phases of cisplatin induced vomiting.[258] Thus, the role of DA in chemotherapy-induced vomiting is not just of mere historical importance, and the shrew data support the current clinical observations that dopamine D_2 receptor antagonists may continue to have a protective role in the control of chemotherapy-induced vomiting.[259]

Although practically all antiemetic regimens contain a glucocorticoid (e.g., dexamethasone) when employed against highly emetogenic chemotherapeutics, often detailed discussion of emetic mechanisms of affected inflammatory eicosanoids such as prostanoids and leukotrienes has not occurred in the literature. In section 7.7 of the current review, some of the pertinent literature regarding the role of prostaglandins and leukotrienes in emesis in general and in chemotherapy-induced vomiting in particular has been discussed. Indeed, it is known that: (1) cisplatin raises tissue levels of eicosanoid inflammatory agents, (2) these increases are associated with emesis, and (3) inhibition of prostaglandin synthesis by indomethacin can prevent cisplatin induced emesis in some emesis models. However, inhibition of prostanid synthesis may not be the major antiemetic mechanism of dexamethasone, which probably involves direct activation of corticosteroid receptors. In addition, cisplatin induced emesis leads to increases in 5-HT or SP turnover in central and peripheral emetic loci, and the COX inhibitor indomethacin is known to reduce both SP signaling and intestinal 5-HT release, which may represent indirect antiemetic actions of dexamethasone. As discussed above, dexamethasone possesses both central and peripheral antiemetic activity as well as having some efficacy against both early- and delayed phases of cisplatin induced vomiting (see section 9.2.3). Alterations in other inflammatory mediator functions have also been suggested.[150]

9.2. Efficacy of Antiemetics against Chemotherapy-Induced Vomiting

9.2.1. Dopamine D_2 Receptor Antagonists

On the basis of initial findings in animal models, clinical studies in the past 3 decades have further refined the utility

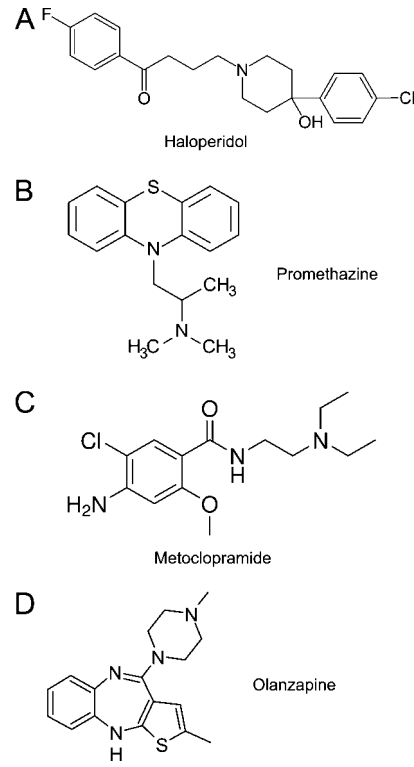

Figure 9. Structural relationships of emesis-modulating, dopamine receptor-binding compounds. Dopamine D_2 receptor antagonism has been associated with the antiemetic effects of these compounds. (A) The butyrophenones, exemplified by haloperidol, were used as antiemetics but are more frequently used as antipsychotics due to their potent psychoactive effects. (B) Another class of D_2 antagonists, the phenothiazines, is represented by promethazine. It has significant histaminergic activity in addition to its dopaminergic antagonist activity, and causes sedation and other histaminergically related side effects at antiemetic doses. (C) Metoclopramide, a modified benzamide compound, has mixed D_2 and 5-HT_3 antagonist activity and was a first-line antiemetic until the advent of the "setron" classes of 5-HT_3 antagonist antiemetics (see figure 4). (D) The atypical antipsychotic olanzapine, a heavily modified benzodiazepine derivative, has activity at both dopaminergic and serotonergic receptors and has been used as an effective antiemetic against moderately emetogenic chemotherapeutic agents.

of modern antiemetics. Ideally, the purpose of antiemetic therapy is to completely prevent nausea and vomiting. Although high-dose cisplatin-type therapy causes vomiting in virtually all treated patients, basic and clinical research has led to steady improvements in control of chemotherapy-induced vomiting. Today vomiting can be completely prevented in up to 70−80% of cancer patients receiving chemotherapy. This success began with the clinical use of of dopamine D_2 antagonists (presented in Figure 9) such as butyrophenones (e.g., haloperidol; Figure 9A), phenothiazines (e.g., promethazine; Figure 9B), and substituted benzamides (e.g., metoclopramide; Figure 9C).[455] However, these agents are generally characterized by their low antiemetic efficacy (10−30% complete protection) and greater potential for side effects. Likewise, selective dopamine D_2 antagonists fail to prevent acute emesis following high-dose cisplatin administration in several animal models of emesis.[118,456,457] Thus, in current practice, phenothiazines are used either as prophylaxis in patients receiving chemotherapy with low emetogenic potential or as a salvage antiemetic in patients who experience breakthrough emesis. Butyrophenones and standard doses of metoclopramide have a similar spectrum of clinical utility, although high doses of metoclopramide have

improved efficacy due to their 5-HT_3 antagonist properties. While addition of dexamethasone has been shown to potentiate the antiemetic potential of dopamine D_2 antagonists in the acute phase,[2] low-dose metoclopramide (DA antagonism) can enhance the overall efficacy of standard antiemetic therapy (a 5-HT_3 antagonist and dexamethasone) against high-dose cisplatin exposure.[458] Indeed, more recently, the atypical antipsychotic olanzapine has been introduced (Figure 9D), which has antagonist properties against several emetic neurotransmitters including DA and 5-HT. It was effective against delayed emesis in cancer patients caused by high (67% complete protection) or moderate (100% complete protection) emetogenic chemotherapy with acceptable toxicity.[459] When combined with standard antiemetic therapy (e.g., a "setron" 5-HT_3 antagonist plus dexamethasone), the combination therapy was significantly more effective in controlling acute (100%) and delayed (80%) emesis.[460,461] These findings point to the importance of DA release in both the gastrointestinal tract and brainstem emetic loci reported recently in the least shrew during peak immediate and delayed phases of cisplatin induced vomiting.[258] In addition, based on the discussed basic studies, both central and peripheral dopaminergic mechanisms may play roles in chemotherapy-induced vomiting.

9.2.2. Cannabinoids

Over 40 clinical trials were carried out in the 1970s−1980s to evaluate the antiemetic potential of cannabinoids against chemotherapy-induced vomiting.[301,303] Most studies used a crossover design, and in each study the sample size was small, varying from less than 25 to over 100. In addition, a number of these studies had included patients who had not responded to the antiemetic treatment with available conventional dopamine D_2 antagonists. Phyto- and synthetic cannabinoids appear to be slightly superior to conventional DA antagonists since complete protection against nausea or vomiting can be achieved in about 37−57% of patients. In addition, Δ^8-THC was effective during the entire duration of chemotherapy-induced vomiting in children suffering from different types of malignancies,[315] while Δ^9-THC has been shown to suppress vomiting at peak phases of both acute and delayed cisplatin induced emesis in least shrews.[93] However, cannabinoids' antiemetic usefulness in the clinic is limited by the high incidence of problematic side effects such as dizziness, dysphoria, and hallucinations. On the other hand, other side effects such as sedation and euphoria can be beneficial. Thus, dronabinol is currently used in the treatment of breakthrough or refractory chemotherapy-induced vomiting.[259,301] Combination regimens containing either Δ^9-THC or synthetic cannabinoids with standard D_2 antagonists have not been extensively studied either in animal models or in the clinic. From clinical trials it appears that such combined regimens are more advantageous than each antiemetic alone, since in some trials cannabinoids' side effects were attenuated, while their antiemetic efficacy was either potentiated or unaffected by DA antagonists.[301] Only one limited clinical study has investigated the antiemetic efficacy of Δ^9-THC in combination with dexamethasone and ondansetron against chemotherapy-induced vomiting, and the results indicated no potentiation.[322] These results were further confimed in the least and house musk shrews, since only low but not high doses of Δ^9-THC potentiate the antiemetic effects of low and not larger doses of 5-HT_3 antagonists against acute cisplatin induced vomiting (for further discussion, see section 6.5). Furthermore, no additive or synergistic interaction has been found between Δ^9-THC and dexamethasone against acute emesis following high-dose cisplatin exposure in the least shrew.[323] However, a small randomized clinical trial has shown that dexamethasone enhances the antiemetic potential of the synthetic cannabinoid nabilone in lung cancer patients receiving cisplatin and other chemotherapeutics.[3] This potentiation in humans but lack of additive antiemetic effect in least shrews probably reflects the high cisplatin dose used in the animal study. Overall, the earlier discussion indicates that phyto- and synthetic cannabinoids are centrally and peripherally acting, broad-spectrum, CB_1 agonist antiemetics, which are effective against diverse emetogens including the acute and delayed phases of cisplatin induced vomiting in several animal models of emesis, as well as against chemotherapy-induced vomiting in humans. On the other hand, endocannabinoids possess both emetic and antiemetic properties, and these effects can be modulated by indirect cannabinoid agonists such as selective inhibitors of their reuptake or metabolism (see section 6.5).

9.2.3. Corticosteroids

A meta-analysis of randomized evidence shows that corticosteroids such as dexamethasone offer a clear advantage over placebo for protection against vomiting induced in both acute and delayed phases in cancer patients receiving chemotherapy.[462] Dexamethasone was among the first antiemetics to be introduced in the clinic against chemotherapy-induced vomiting and is currently used extensively, especially in combination with other antiemetics. Approximately six patients need to be treated to prevent one patient from experiencing emesis in either chemotherapy-induced vomiting phase. Clinical antiemetic efficacy in terms of complete response rates is improved by 15−20% when dexamethasone is added to 5-HT antagonists in both phases of chemotherapy-induced vomiting.[431] The available data further suggest superiority of dexamethasone over a 5-HT_3 antagonist in direct comparison for protection against delayed emesis. These findings are supported by preclinical studies, in that dexamethasone and other glucocorticoids generally attenuate both phases of cisplatin induced vomiting in animals, and when combined with a 5-HT_3 antagonist, additive efficacy is observed during both phases.[395,463,464] Although there is no head-to-head clinical trial comparing the antiemetic effects of dexamethasone against an NK_1 antagonist during chemotherapy-induced vomiting, addition of aprepitant increases the antiemetic efficacy of a standard chemotherapy regimen (dexamethasone + a 5-HT_3 antagonist) by about 20%.[431,465] In addition, only one published animal study in the acute setting has investigated the effect of a combination of an NK_1 antagonist with dexamethasone, and the results showed significant additive antiemetic efficacy relative to each drug alone, which supports the above clinical findings.[466] Corticosteroids are sometimes underutilized because of potential for side effects. However, the dose of dexamethasone used varies across studies without variability in the antiemetic effect, and thus lower doses may be adequate for achieving the protective effect.[462] Significant basic evidence suggests both peripheral and central mechanisms are at play in the antiemetic actions of dexamethasone and related antiemetics (see section 7.7).

9.2.4. Serotonin 5-HT$_3$ Receptor Antagonists

Development of 5-HT$_3$ receptor antagonists has been one of the most significant advances in cancer chemotherapy. As single agents, 5-HT$_3$ antagonists (ondansetron, granisetron, tropisetron, dolasetron, and palonosetron; see Figure 4C−E) have response rates of 60−80% as antiemetics in cancer patients and have dramatically improved the management of acute chemotherapy and radiotherapy.[259,430,431,455] 5-HT$_3$ antagonists appear to be more advantageous than DA antagonists, dexamethasone, the NK$_1$ antagonists, and phyto- and synthetic cannabinoids in the acute-phase chemotherapy-induced vomiting in both highly and moderately emetogenic chemotherapies. Oral forms of 5-HT$_3$ antagonists are as effective as intravenous preparations, which are well-tolerated and cause few side effects other than headache. Summary of several meta-analyses of published clinical trials indicate no clear advantage of one setron over the other and, even if significant differences existed, it is difficult to know whether these differences are clinically relevant. Although the long acting and potent 5-HT$_3$ antagonist palonosetron has outperformed ondansetron and dolasetron in several secondary and subgroup analyses in head-to-head comparisons, the primary end point (i.e., no emesis) leads to relative noninferiority of any of the setrons.[465] Basic studies in different animal models of acute and delayed emesis support the clinical findings, in that 5-HT$_3$ receptor antagonists are mainly effective antiemetics in the immediate phase while dexamethasone potentiates their antiemetic efficacy during both phases.[434,436]

9.2.5. Neurokinin NK$_1$ Receptor Antagonists

Only preliminary studies involving small numbers of patients have evaluated the antiemetic effects of aprepitant in isolation against chemotherapy-induced vomiting. In general, vofopitant, CP122,721, or L-758,298 were not particularly effective when given as monotherapy to cisplatin exposed cancer patients prior to immediate or delayed emesis.[231] Thus, unlike the discussed potent antiemetic efficacy of NK$_1$ receptor antagonists against both phases of cisplatin induced emesis in animal models, these preliminary studies show that NK$_1$ antagonists by themselves are not effective against cisplatin induced emesis in cancer patients, but they can potentiate the antiemetic efficacy of standard therapy (a 5-HT$_3$ antgonist plus dexamethasone) against both acute and delayed chemotherapy-induced vomiting.[210,259,395] Indeed, recent pooled results from two large clinical trials show 20% overall improvement in emesis protection ($p < 0.001$) for the 5-day period after cisplatin exposure by combination regimens containing the NK$_1$ antagonist aprepitant with ondansetron and dexamethasone, compared with the standard regimen of ondansetron and dexamethasone.[467] In addition, aprepitant caused a 13% ($p < 0.001$) improvement in the prevention of acute emesis as well as a 21% ($p < 0.01$) improvement in the delayed phase. Animal studies support these efficacy enhancements in patients, since addition of a 5-HT$_3$ antagonist or dexamethasone also potentiates the antiemetic efficacy of the NK$_1$ antagonist in cisplatin treated ferrets.[466] On the basis of the latter clinical findings, the most recent antiemetic guideline in oncology by the American Society of Clinical Oncologists (ASCO) considers 5-HT$_3$ antagonists to be no more effective than other agents (aprepitant, dexamethasone, or prochlorperazine) during the delayed phase, and thus are not universally considered as standard therapy in patients receiving highly emetogenic chemotherapy.[259,465] This conclusion was mainly based upon (1) incomplete 5-HT turnover studies during delayed emesis in humans (see section 9.1) and (2) results indicating that regimens consisting of different 5-HT$_3$ receptor antagonists combined with dexamethasone are not superior to dexamethasone alone.[465] However, on the basis of the discussed newly published basic and clinical turnover studies involving 5-HT, DA, and SP during both emetic phases and a lack of comparative clinical trials containing combinations of a 5-HT$_3$ antagonist, an NK$_1$ antagonist, dexamethasone, and a D$_2$ antagonist (e.g., olanzapine) throughout the entire duration of chemotherapy-induced vomiting, the previous, relatively firm conclusion should be open to modification.

9.2.6. Chemotherapy and Nausea

Cancer patients receiving chemotherapy experience not only emesis but also nausea,[468] which is a continuous feeling of gastric discomfort, rather than the episodic discomfort of vomiting. Since nausea is a subjective sensory experience, indirect measures such as salivation or chewing have been proposed as indices of nausea-like activity in animals.[436] Retching behavior is continuous as well as biphasic, with broadened peaks coinciding with the immediate and delayed vomit peaks in animals, and which could probably reflect initial events soon after sensation of nausea.[258] Indeed, involvement of disturbed gastrointestinal motility in the genesis of nausea has been suggested.[469] However, salivation, chewing, and retching behaviors are usually considered part of the prodromal phase (events that precede emetic episodes) run by the motor program in the emetic circuitry, and may not be related to "nauseous" circuits. Whatever the case, the current antiemetics do not alleviate nausea in patients receiving chemotherapy, although aprepitant-like agents show promise. In fact, one study reported that a measure of the effect of nausea and vomiting on daily life had shown a significantly higher percentage of patients reporting "minimal or no impact of chemotherapy-induced vomiting on daily life" in the aprepitant arm containing a 5-HT$_3$ antagonist plus dexamethasone, versus the standard group lacking the NK$_1$ antagonist in patients receiving highly emetogenic chemotherapy.[467] Furthermore, apart from the percentage of patients with no nausea on day 1, all end points related to nausea showed a statistically significant difference in favor of aprepitant. In addition, the absolute differences for the nausea-related outcomes during the entire duration of chemotherapy-induced vomiting (no nausea, no significant nausea, and the nausea domain) showed a modest but consistent impact of aprepitant in reducing nausea. NK$_1$ antagonists by themselves also appear to demonstrate efficacy against postoperative vomiting, but not the associated nausea in patients recovering from surgery.[210,260] Likewise, NK$_1$ antagonists such as GR205171 are not effective against motion-induced nausea in humans.[210,262] Thus, either neurotransmitter and/or anatomical substrates of nausea for these diverse conditions are different, or only the combination regimen containing aprepitant, a 5-HT$_3$ antagonist and dexamethasone, culminates in efficacy against nausea reported in chemotherapy.[467]

10. Conclusions

The main goal of this review was to bridge some of the existing gaps in the literature and more critically evaluate the basis of central/peripheral components of chemotherapy-induced vomiting neurotransmitter dogma. Although the discussed findings are important breakthroughs in both basic science and clinical oncology emesis research, the incidence of nausea and vomiting still remains unacceptably high and is a major factor in premature discontinuation of chemotherapy. Our inability to develop more effective antiemetic regimens against chemotherapy-induced vomiting is due to a partial appreciation of relative temporal and spatial contributions of multiple emetic neurotransmitters (DA, 5-HT, SP, eicosanoids such as prostaglandins, leukotrienes, and endocannabinoids, and related downstream emetic metabolites) having differential and overlapping sequential release and interplay, in the regulation of both phases of chemotherapy-induced vomiting, in both the brainstem and the gastrointestinal tract. The discussed clinical evidence is supportive of this notion since no single antiemetic is completely effective at blocking emesis in either phase, but when administered together, the antiemetic efficacy of the combination is greater than that of each agent given individually. The future challenge is to build upon the current basic science evidence for these changes in the turnover of the cited emetic neurotransmitters, their receptors, and downstream signal transduction mechanisms during the full time course of chemotherapy-induced vomiting. In this way, more comprehensive and concomitant multineurotransmitter turnover measurements in patients could be made, which would lead to utilization of more broad-spectrum antiemetic regimens for the prevention of chemotherapy-induced vomiting in the clinic.

11. Abbreviations

2-AG	2-arachidonoylglycerol
5-HIAA	5-hydroxyindoleacetic acid
5-HPETE	5-hydroperoxyeicosatetraenoic acid
5-HT	5-hydroxytyramine (serotonin)
5-HTP	5-hydroxytryptophan
5-HTQ	Serotonin, quaternary ionic form (*N*,*N*,*N*-trimethylserotonin)
AA	arachidonic acid
AP	area postrema
cAMP	cyclic AMP
CGRP	calcitonin gene-related peptide
CNS	central nervous system
COMT	catechol-*O*-methyltransferase
COX	cyclooxygenase
CTZ	chemoreceptive trigger zone
DA	dopamine
DMNX	dorsal motor nucleus of the vagus
ENS	enteric nervous system
EPI	epinephrine
EPSP	excitatory postsynaptic potential
FAAH	fatty acid amide hydrolase
GI	gastrointestinal
HVA	homovanillic acid
ICC	interstitial cells of Cajal
IPAN	intrinsic primary afferent neuron
L-DOPA	L-3,4-dihydroxyphenylalanine
LO	lipoxygenase
MAGL	monoacylglycerol lipase
MAO	monoamine oxidase
mCPBG	*m*-chlorophenylbiguanide
NADA	*N*-arachidonoyldopamine
NE	norepinephrine
NKA	neurokinin A
NKB	neurokinin B
(m)NTS	nucleus of the solitary tract (medial subnucleus)
PL	phospholipases
PPT1	preprotachykinin-1
SP	substance P
SSP-SAP	stable substance P-saporin
TPH	tryptophan hydroxylase
VIP	vasoactive intestinal peptide
Δ^8-THC	delta-8-tetrahydrocannabinol
Δ^9-THC	delta-9-tetrahydrocannabinol

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