Chapter 6

Role of Calcium in Vomiting

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http://dx.doi.org/10.5772/intechopen.78370

Abstract

Cisplatin-like chemotherapeutics cause vomiting via calcium (Ca^{2+})-dependent release of multiple neurotransmitters/mediators (dopamine, serotonin, substance P, prostaglandins and leukotrienes) from the gastrointestinal enterochromaffin cells and/or the brainstem. Intracellular Ca^{2+} signaling is triggered by activation of diverse emetic receptors (including neurokininergic NK_1, serotonergic 5-HT_3, dopaminergic D_2, cholinergic M_1, or histaminergic H_1), whose stimulation in vomit-competent species evokes emesis. Other emetogens such as cisplatin, rotavirus NSP4 protein, and bacterial toxins can also induce intracellular Ca^{2+} elevation. Our findings demonstrate that application of the L-type Ca^{2+} channel (LTCC) agonist FPL 64176 and the intracellular Ca^{2+} mobilizing agent thapsigargin (a sarco/endoplasmic reticulum Ca^{2+}-ATPase inhibitor) cause vomiting in the least shrew. On the other hand, blockade of LTCCs by corresponding antagonists (nifedipine or amlodipine) not only provide broad-spectrum antiemetic efficacy against diverse agents that specifically activate emetogenic receptors such as 5-HT_3, NK_1, D_2, and M_1 receptors, but can also potentiate the antiemetic efficacy of palonosetron against the nonspecific emetogen, cisplatin. In this review, we will provide an overview of Ca^{2+} involvement in the emetic process; discuss the relationship between Ca^{2+} signaling and the prevailing therapeutics in control of vomiting; highlight the current evidence for Ca^{2+}-signaling blockers/inhibitors in suppressing emetic behavior and also draw attention to the clinical benefits of Ca^{2+}-signaling blockers/inhibitors for the treatment of nausea and vomiting.

Keywords: cisplatin, vomiting, antiemesis, Ca^{2+}, L-type Ca^{2+} channel

1. Introduction

Acute (≤24 h) and delayed (>24 h) phases of chemotherapy-induced nausea and vomiting cause distressing side-effects which affect the well-being and quality of life of cancer patients

receiving chemotherapy, especially cisplatin [1]. Major neurotransmitter mechanisms underlying chemotherapy-induced nausea and vomiting have been subject of considerable research over the past 45 years. As presented in brief in **Figure 1**, cancer chemotherapeutics such as cisplatin evoke vomiting via local release of a variety of emetic neurotransmitters/mediators (including dopamine, serotonin (5-HT), substance P, prostaglandins and leukotrienes) both from the enterochromaffin cells of the gastrointestinal tract and the brainstem emetic loci in the dorsal vagal complex containing the nucleus tractus solitarius, the dorsal motor nucleus of the vagus and the area postrema [2–4]. The area postrema and the nucleus tractus solitarius contain large numbers of fenestrated capillaries which lack blood-brain barrier and permit neurons in both areas access to blood-borne circulating factors including emetogens [5]. The chemoreceptor trigger zone, in the area postrema has high concentrations of emetic receptors for serotonin ($5\text{-}HT_3$), dopamine ($D_{2/3}$), neurokinin (NK_1), and opioids (μ), among others [2]. Direct stimulation of these receptors in the chemoreceptor trigger zone by emetogens is one important mechanism by which vomiting can occur [6]. The nucleus tractus solitarius receives emesis-related information from the area postrema as well as the gastrointestinal tract conveyed by vagal afferents. The dorsal motor nucleus of the vagus receives axonal projections from nucleus tractus solitarius [7] and sends emetic signals via motor efferent pathways to the gastrointestinal tract and modulates vomiting behaviors [2, 5, 8, 9] (**Figure 1**). In addition, chemotherapeutic drugs may evoke release of emetic neurotransmitters/mediators from the gastrointestinal tract into the blood to be directly delivered to the area postrema via a

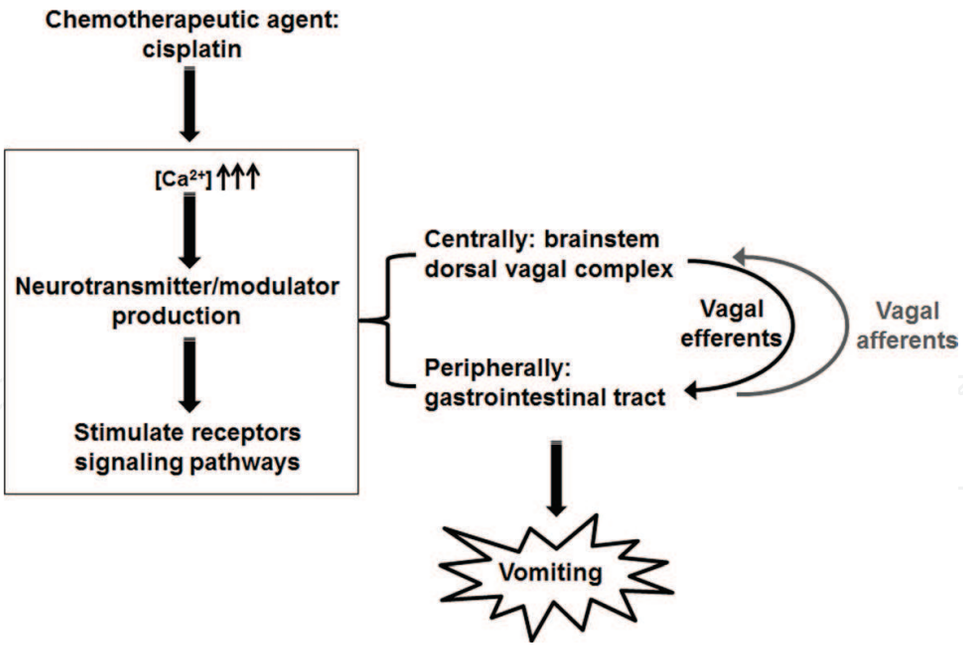

Figure 1. Brief illustration of the mechanisms underlying vomiting induced by chemotherapeutic agent cisplatin. Mechanisms underlying cisplatin-induced vomiting can be simplified as: (1) cisplatin can increase cytoplasmic Ca^{2+} level to evoke Ca^{2+}-dependent release of emetic neurotransmitters/mediators at the brainstem emetic loci, the dorsal vagal complex, and subsequently activates diverse receptors and their corresponding signaling pathways. These emetic signals are output to the gastrointestinal tract via efferents to trigger vomiting [2, 4–9]; (2) cisplatin-induced peripheral release of neurotransmitters/mediators from the gastrointestinal tract into the blood can directly stimulate the dorsal vagal complex, activate receptors signaling pathways and trigger vomiting [2, 10]; and (3) the peripherally-released emetic neurotransmitters/mediators stimulate their corresponding receptors present on vagal afferents in the gastrointestinal tract which indirectly activate brainstem emetic nuclei and trigger vomiting [6].

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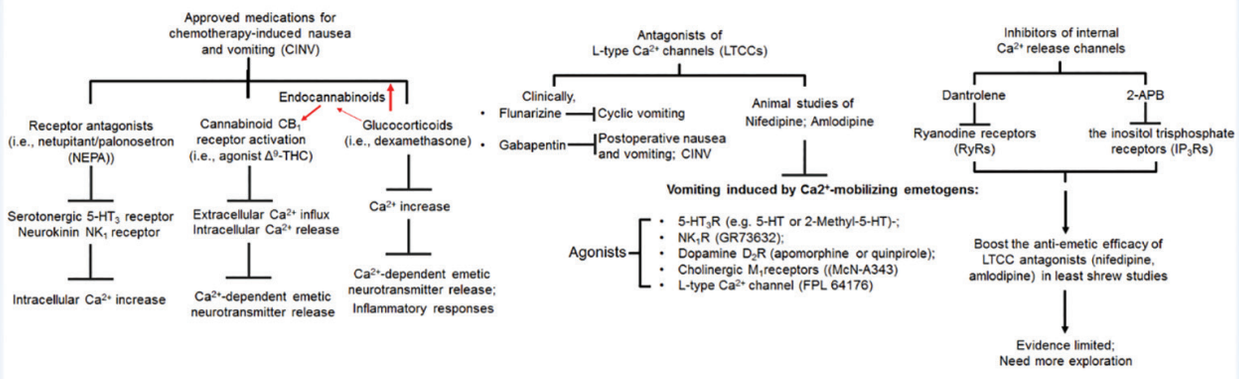

Figure 2. Overview of evidence for suppression of Ca^{2+} signaling involved in anti-vomiting actions of antiemetic agents. (1) Netupitant and palonosetron are highly selective respective antagonists of NK_1Rs and $5\text{-}HT_3Rs$ are approved to treat the acute- and delayed- phases of chemotherapy-induced nausea and vomiting (CINV) in cancer patients [79–82]. Our studies [83–86] indicate that suppression of Ca^{2+} signaling is involved in antiemetic efficacy of both palonosetron and netupitant. (2) Cannabinoids such as delta-9-tetrahydrocannabinol exert their antiemetic efficacy via direct activation of CB_1 receptors (CB_1R) [92, 94, 98–100]. The ability of CB_1R agonists to suppress both extracellular Ca^{2+} influx [111–115] and intracellular Ca^{2+} release from the sarco/endoplasmic reticulum stores [15, 117], result in inhibition of Ca^{2+}-dependent neurotransmitter release [108] and is probably the fundamental mechanisms underlying the antiemetic efficacy of CB_1R cannabinoid agonists against CINV [95–97]. (3) Glucocorticoids such as dexamethasone reduce both acute and delayed CINV [6]. Glucocorticoids' ability to decrease the abnormal elevation of cytosolic Ca^{2+} concentration [122], and subsequently control Ca^{2+}-dependent neurotransmitter release [6, 121, 126] and inflammatory responses [6]. Increased release of endocannabinoids and subsequent CB_1R activation may also be involved in antiemetic actions of glucocorticoids [123–125]. (4) The L-type Ca^{2+} channel (LTCC) antagonist flunarizine can reduce cyclic vomiting in patients [151, 152]. Gabapentin binds to the alpha-2/delta auxiliary subunits of LTCCs, and exerts inhibitory actions on trafficking and activation kinetics of LTCCs [153]. Gabapentin can be used as an anti-nausea and antiemetic agent in postoperative nausea and vomiting [154, 155] and in CINV [156, 157]. (5) LTCC antagonists (nifedipine and amlodipine) are broad-spectrum antiemetics when delivered systemically against diverse specific and nonselective emetogens. (6) Suppression of intracellular Ca^{2+} release from the sarco/endoplasmic reticulum through the inositol trisphosphate receptors (IP_3Rs) and ryanodine receptors (RyRs) may be additional targets for the prevention of nausea and vomiting, since functional and physical linkages between Ca^{2+} channels on cell membrane and IP_3Rs/RyRs play a role in Ca^{2+} signaling [160–166]. In the least shrew emesis model, the RyRs antagonist dantrolene can potentiate the antiemetic efficacy of amlodipine against $5\text{-}HT_3R$ agonist 2-Methyl-5-HT-induced vomiting [25]; and dantrolene together with the IP_3R antagonist 2-APB can potentiate the antiemetic efficacy of nifedipine against thapsigargin-induced vomiting [70].

blood-borne pathway which then triggers vomiting [2, 10], and/or the released emetic neurotransmitters/mediators stimulate their corresponding receptors present on vagal afferents in the gastrointestinal tract which indirectly activate brainstem emetic loci primarily in the nucleus tractus solitaries to trigger vomiting [6].

Ca^{2+} is not only one of the most universal and versatile signaling molecules, it is also an extremely important factor in both the physiology and pathology of living organisms. At rest, diverse cells have strict and well-regulated mechanisms to maintain low nM cytosolic Ca^{2+} levels [11]. Cytoplasmic Ca^{2+} concentration is a dominant factor in determining the amount of transmitter released from nerve terminals [12]. Thus, Ca^{2+} mobilization can be an important aspect of vomit induction since it is involved in both triggering the quantity of neurotransmitter released coupled with receptor activation, as well as post-receptor excitation-transcription coupling mechanisms [13]. Studies using Ca^{2+} imaging performed in vitro in the brainstem slice preparation suggest that emetic agents evoke direct excitatory effects on cytosolic Ca^{2+} signals in vagal afferent terminals in the nucleus tractus solitarius which potentiate local neurotransmitter release [5, 14, 15]. Therefore, chemotherapeutics including cisplatin seem to activate emetic circuits through a number of neurotransmitters released in a Ca^{2+}-dependent

manner in specific vomit-associated neuroanatomical structures. In both the periphery and the brainstem, emetic neurotransmitters/mediators—such as acetylcholine, dopamine, 5-HT, substance P, prostaglandins, leukotrienes, and/or histamine—may act independently or in combination to evoke vomiting after cisplatin administration [16] (**Figure 1**). In this review, we focus on the current evidence supporting the significance of Ca^{2+} signaling in emesis generation and its relationship to antiemetic efficacy, as well as the corresponding development of potential novel antiemetic medications, as shown in brief in **Figure 2**.

2. Emerging roles of Ca^{2+} in emesis

2.1. Emetic receptor stimulation increases intracellular Ca^{2+} concentration

Excitatory receptor activation by corresponding agonists can increase cytosolic Ca^{2+} levels via both mobilization of intracellular Ca^{2+} stores (e.g., endoplasmic reticulum = ER) and influx from extracellular fluid [17]. The evoked cytoplasmic Ca^{2+} increase may result from direct activation of ion channels, or indirectly via signal transduction pathways following G protein-coupled receptor activation. The neurokinin NK1 receptor (NK_1R) is a member of the tachykinin family of G-protein-coupled receptors. NK_1R stimulation by substance P or corresponding selective agonists such as GR73632, can increase cytosolic Ca^{2+} concentration. In fact GR73632-induced activation of NK_1Rs can evoke intracellular Ca^{2+} release from the sarco/endoplasmic reticulum stores via Gα/q-mediated phospholipase C pathway, which subsequently evokes extracellular Ca^{2+} influx through L-type Ca^{2+} channels (LTCCs) [17–19]. The serotonergic 5-HT_3 receptor (5-HT_3R) is a Ca^{2+}-permeable ligand-gated ion channel [20]. Cell lines studies have demonstrated that activation of 5-HT_3Rs by 5-HT or its analogs can evoke extracellular Ca^{2+} influx into cells in a manner sensitive to both 5-HT_3R antagonists (tropisetron, MDL7222, metoclopramide) and LTCC blockers (verapamil, nimodipine, nitrendipine) [20–24]. These studies suggest that both L-type- and 5-HT_3-receptor Ca^{2+}-permeable ion channels are involved in extracellular Ca^{2+} influx evoked by 5-HT_3R agonists. Moreover, 5-HT_3R activation indirectly causes release of Ca^{2+} from ryanodine-sensitive intracellular Ca^{2+} stores subsequent to the evoked extracellular Ca^{2+} influx which greatly amplifies the cytoplasmic concentration of Ca^{2+} [23]. In fact, our findings from behavioral studies in the least shrew emesis model [25] further support the notion of Ca^{2+}-induced Ca^{2+} release following 5-HT_3R stimulation, which will be discussed in more detail in Section 3.4. Other emetogens such as agonists of dopamine D_2- [26, 27], cholinergic M_1- [28, 29], histaminergic H_1- [30, 31], and opiate μ- [32, 33] receptors, as well as cisplatin [34], prostaglandins [35, 36], rotavirus NSP4 protein [37, 38] and bacterial toxins [39, 40] also possess the potential to mobilize Ca^{2+} which involve extracellular Ca^{2+} influx and/or Ca^{2+} release from intracellular Ca^{2+} pools. Much of the discussed evidence has been acquired from isolated cells.

The least shrew is an emesis-competent mammal whose reactions to common emetogens are well-defined and correlate closely with human responses [2]. 2-Methyl-5-HT is a well-known selective emetic agonist targeting the emesis-prone 5-HT_3Rs [4]. This vomit-competent species is an excellent animal model for studying the emetic activity of diverse agents [2]. In fact least shrews exhibit dose-dependent full emetic responses to intraperitoneal administration of

both the peripherally-acting 5-HT, as well as to its central nervous system-penetrating analog, 2-Methyl-5-HT [4, 41, 42]. In our studies, incubation of least shrew brainstem slices containing the dorsal vagal complex emetic loci with 2-Methyl-5-HT, results in a rapid increase in intracellular Ca^{2+} concentration as reflected by an increase in fluo-4 AM fluorescence intensity in a palonosetron (a 5-HT_3R antagonist)/nifedipine-sensitive manner [22, 25].

2.2. Emetic potential of Ca^{2+} channel activators: behavioral and immunohistochemical evidence

A variety of Ca^{2+}-permeable ion-channels mediating extracellular Ca^{2+} influx are present in the plasma membrane. Among them are voltage-gated LTCCs, which can be activated by membrane depolarization, and serve as the principal route of Ca^{2+} entry in electrically excitable cells such as neurons and muscle [43, 44]. Recently we have acquired direct evidence for the proposal that Ca^{2+} mobilization is an important facet in the mediation of emesis. In fact we have identified the novel emetogen FPL64176 (**Figure 2**), a selective agonist of LTCCs, which causes vomiting in the least shrew in a dose-dependent manner [45, 46]. All tested shrews vomited at a 10 mg/kg dose of FPL64176 administered intraperitoneally (i.p.). LTCCs have been shown to be present in enterochromaffin cells of guinea pig and human small intestinal crypts [47]. Furthermore, in these cells FPL64176 not only can enhance cytosolic Ca^{2+} concentration, but also increases 5-HT release from enterochromaffin cells [47]. The latter findings may have underpinnings for the mechanisms underlying FPL64176-evoked vomiting observed in least shrew model of emesis. FPL64176 (10 mg/kg., i.p.) can cause Ca^{2+}-dependent 5-HT release from shrew intestinal enterochromaffin cells which in turn could increase vagal afferent activity via stimulation of 5-HT_3 receptors, thereby indirectly triggering emetic signals in the brainstem [2, 48].

Our most recent work has focused on the Ca^{2+}-mobilizing agent thapsigargin (**Figure 3**), a specific and potent inhibitor of the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pump which transports the free cytosolic Ca^{2+} into the lumen of the sarco/endoplasmic reticulum to

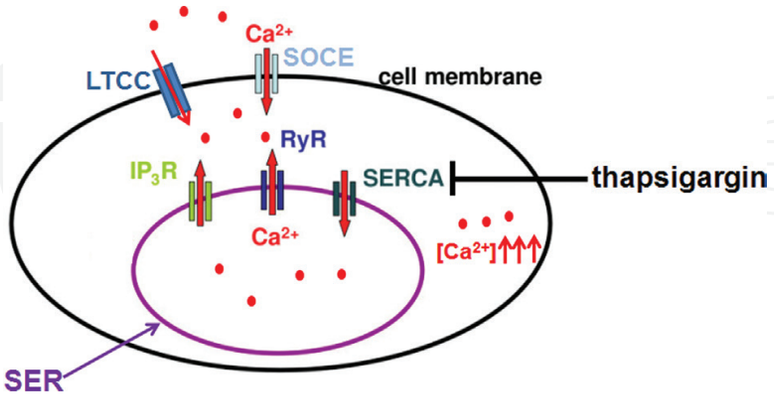

Figure 3. A schematic representation of extracellular Ca^{2+} influx and intracellular Ca^{2+} release contributing to thapsigargin-elicited Ca^{2+} mobilization. Intracellular Ca^{2+} release from the sarco/endoplasmic reticulum (SER) Ca^{2+} stores through the inositol triphosphate receptors (IP_3Rs) and ryanodine receptors (RyRs) is counter-balanced by continuous Ca^{2+} uptake from the cytoplasm into SER stores by the SER Ca^{2+}-ATPase pump (SERCA). Thapsigargin is a specific inhibitor of SERCA and thus enhances cytosolic levels of Ca^{2+}, a process involving SER Ca^{2+} release via IP_3Rs and RyRs as well as extracellular Ca^{2+} entry through Ca^{2+} channels located in the plasma membrane including store-operated Ca^{2+} channels (SOCE) and L-type Ca^{2+} channels (LTCCs) [49–60].

counter-balance the cytosolic intracellular Ca^{2+} release from the sarco/endoplasmic reticulum into the cytoplasm via the inositol trisphosphate receptors (IP_3Rs) and ryanodine receptors (RyRs) [49–51]. Thapsigargin also causes intracellular release of stored Ca^{2+} from the sarco/endoplasmic reticulum into the cytosol which subsequently evokes extracellular Ca^{2+} influx predominantly through store-operated Ca^{2+} entry (SOCE) in non-excitable cells [52–54]. In total, these events lead to a significant rise in the free concentration of cytosolic Ca^{2+} [55–57]. In addition, a partial involvement of LTCCs in thapsigargin-evoked contraction has also been demonstrated in rat stomach smooth muscle cells [58], rat gastric smooth muscle [59], and cat gastric smooth muscle [60]. On the other hand, the potential of thapsigargin as a Ca^{2+}-modulating cancer chemotherapeutic agent has been evaluated in both cells and animal models [61]. Thapsigargin-evoked increases in cytosolic Ca^{2+} concentration can lead to cell apoptosis, which can result in eradication of cancer cells of the breast [62], prostate [63], colon [64] and kidneys [65]. Clinically, a prodrug form of thapsigargin, mipsagargin, is currently under clinical trial as a targeted cancer chemotherapeutic agent with selective toxicity against cancer cells in tumor sites with minimal side-effects to the host [66–69]. In our studies, intraperitoneal administration of thapsigargin (0.1–10 mg/kg) caused vomiting in least shrews in a dose-dependent, but bell-shaped manner, with maximal efficacy at 0.5 mg/kg. An important consideration for the emetic potential of thapsigargin is that it augments the cytosolic levels of free Ca^{2+} in emetic loci as a result of SERCA inhibition as indicated in our latest discussed finding [70], which is the first study to reflect emesis as a major side-effect of thapsigargin when delivered systemically.

c-Fos induction has been used to evaluate differential neuronal activation [71]. Our lab has applied immunostaining and detected c-Fos induction in the brainstem emetic nuclei to demonstrate central responsiveness to peripheral administration of a variety of emetogens [4, 70, 72, 73]. The participation of the central emetic neurons in FPL64176-induced vomiting is further indicated by evoked c-Fos expression in brainstem emetic nuclei, the nucleus tractus solitarius and the dorsal motor nucleus of the vagus (unpublished data). Thus, the blood-brain barrier permeable agent FPL64176 [74–76] could excite emetic neurons directly in the nucleus tractus solitarius and the dorsal motor nucleus of the vagus. Thapsigargin (0.5 mg/kg) also causes increases in c-Fos immunoreactivity in the brainstem emetic nuclei including the area postrema, the nucleus tractus solitarius and the dorsal motor nucleus of the vagus [70].

3. Ca^{2+} intervention mechanisms relevant to antiemetic approaches

3.1. Receptor antagonist antiemetic regimens such as netupitant/palonosetron (NEPA)

The ultimate aim of prophylactic management of chemotherapy-induced nausea and vomiting is to abolish both the acute- and delayed phases of vomiting which will help to improve the well-being and quality of life of cancer patients receiving chemotherapy. Cisplatin-like chemotherapeutics cause release of multiple emetogenic neurotransmitters in both the central nervous system and the gastrointestinal tract and no available single antiemetic administered alone can provide complete efficacy. Significant initial work had suggested that while activation of 5-HT_3Rs by serotonin in the gastrointestinal tract is involved in the mediation of acute phase of chemotherapy-induced nausea and vomiting, the delayed phase is due to stimulation

of NK_1Rs subsequent to release of substance P in the brainstem [77, 78]. However, our more recent findings suggest that 5-HT and substance P are concomitantly involved in both emetic phases in the gastrointestinal tract as well as in the brainstem [2, 16]. While netupitant is a highly selective and a longer-acting second generation NK_1R antagonist, palonosetron is considered as a second generation 5-HT_3R antagonist with a unique antiemetic profile in both humans [79, 80] and the least shrew model of emesis [45]. A successful regimen of an oral fixed combined dose of netupitant/palonosetron (NEPA) (**Figure 2**) has been formulated with over 85% clinical efficacy, good tolerability, and high central nervous system penetrance for the prophylactic treatment of acute and delayed chemotherapy-induced nausea and vomiting in cancer patients receiving chemotherapy [9, 81, 82].

Recent evidence accumulated from HEK293 cells stably transfected with 5-HT_3Rs suggest that suppression of Ca^{2+} signaling is involved in antiemetic efficacy of both palonosetron and netupitant. Indeed, Rojas et al. [83, 84] have shown that palonosetron causes a persistent inhibition of 5-HT_3R function as reflected by a near complete suppression of 5-HT-evoked extracellular Ca^{2+} influx. They have further demonstrated that palonosetron can prevent enhancement of substance P-induced intracellular Ca^{2+} release in response to serotonin in NG108–15 cells expressing both 5-HT_3Rs and NK_1Rs [85]. Our Ca^{2+} monitoring studies performed on acutely-prepared least shrew brainstem slices also demonstrate that palonosetron can abolish enhancement of intracellular Ca^{2+} levels in brainstem slices evoked by the selective 5-HT_3R agonist 2-Methyl-5-HT [25]. The latter finding provides more relevant ex-vivo evidence for the Ca^{2+}-modulating antiemetic effect of palonosetron in a vomit-competent species. The role of netupitant in suppression of substance P-evoked enhancement of intracellular Ca^{2+} levels has also been demonstrated via Ca^{2+} mobilization assays in vitro in CHO cells expressing the human NK_1Rs. Moreover, pronetupitant, an intravenous alternative to the oral netupitant, appears to be more potent than netupitant in both in vitro Ca^{2+} measurement studies and in vivo animal behavioral evaluations of substance P in rats [86]. In addition, another clinically approved NK_1R antagonist antiemetic rolapitant, has been shown to suppress the ability of the selective NK_1R agonist GR73632 to evoke intracellular Ca^{2+} release [9, 87–89]. The discussed findings clearly suggest that Ca^{2+} is a major player in the initiation of vomiting evoked by diverse emetogens.

3.2. Cannabinoid CB_1 receptor agonists

Before the introduction of first generation 5-HT_3R antagonists, several phyto- and synthetic cannabinoids including dronabinol (delta-9-tetrahydrocannabinol, Δ^9-THC (**Figure 2**)), levonantradol and nabilone, were evaluated in cancer patients for suppression of chemotherapy-induced nausea and vomiting that were not effectively controlled by other available antiemetics [2, 90]. Cannabinoids are increasingly being tested as antiemetics against cisplatin-induced emesis in animal experiments using house musk shrews [91], ferrets [92], or least shrews [73, 93]; nausea-related behavior in rats [91]; radiation-induced emesis in the least shrew [94]; as well as both phases of chemotherapy-induced nausea and vomiting in the clinic [95–97]. Cannabinoid agonists exert their antiemetic efficacy via direct activation of CB_1 receptors (CB_1R) since their antiemetic effects were reversed by CB_1R antagonists [92, 94, 98–100]. Significant evidence for a role for CB_2Rs in emesis is currently lacking [101]. The presence of CB_1Rs in the brainstem nuclei involved in emesis has been confirmed, with a high density of CB_1R immunoreactivity in the dorsal motor nucleus of the vagus and the medial subnucleus

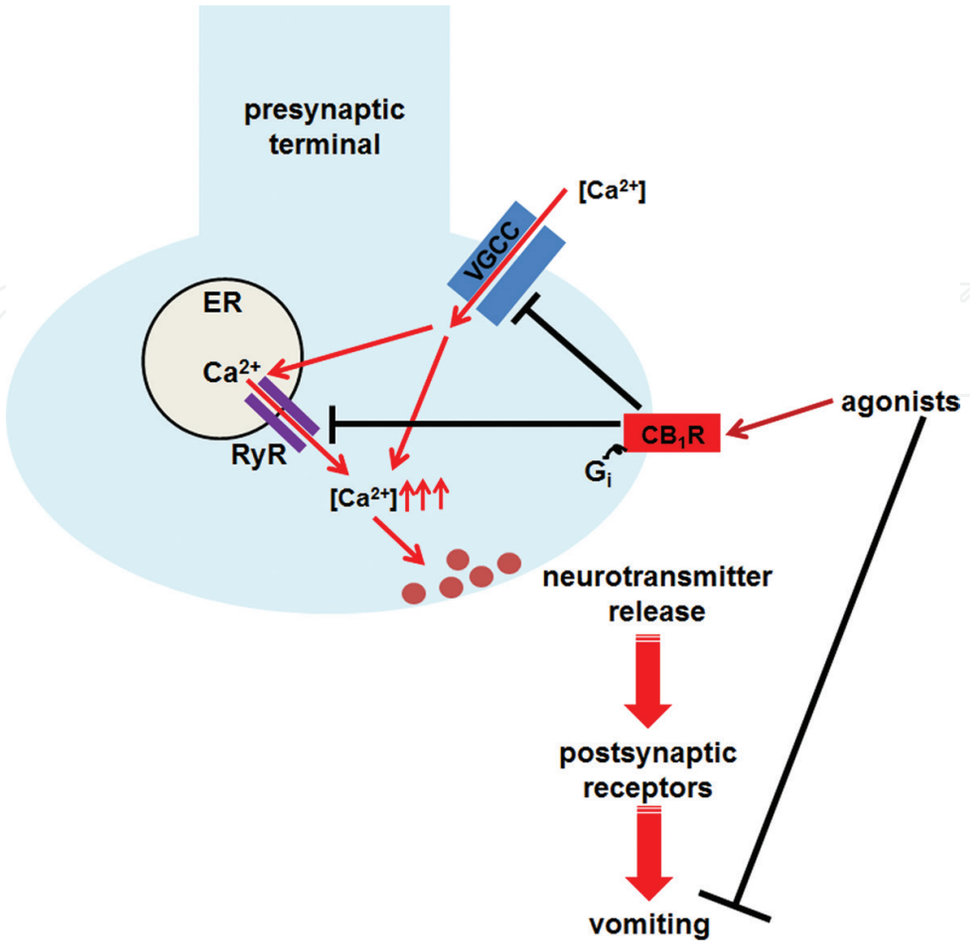

Figure 4. A schematic explanation of the antiemetic action of cannabinoid CB_1R agonists from the perspective of Ca^{2+} signaling. Activation of CB_1R initiates a $G_{i/o}$ mechanism leading to the downregulation of extracellular Ca^{2+} influx through voltage-gated Ca^{2+} channels (VGCCs) as well as endoplasmic reticulum (ER) Ca^{2+} release via ryanodine receptors (RyRs) which has the potential to be activated by extracellular Ca^{2+} entry through VGCCs. The reduction in cytosolic Ca^{2+} attenuates Ca^{2+}-dependent emetic neurotransmitter release, which further results in a reduction in postsynaptic neuronal activation, and ultimately suppression of the vomiting behavior [93, 103, 117].

of the nucleus tractus solitarius, a moderate density in the commissural subnucleus of the nucleus tractus solitarius, and lower densities in the area postrema and dorsal subnucleus of the nucleus tractus solitarius [73, 92]. CB_1R distribution has been also observed in the myenteric plexus of the stomach and duodenum [92]. Furthermore, CB_1Rs have been localized in the myenteric plexus of the rat and guinea pig intestine in nearly all cholinergic neuron terminals [102, 103]. These as well as behavioral evidence [42] suggest that the antiemetic action of cannabinoids involve both the central dorsal vagal complex and intestinal emetic loci. In addition, primary cultures of guinea-pig myenteric neurons express CB_1Rs and exogenously added cannabinoids suppress their neuronal activity, synaptic transmission and mitochondrial transport along axons [104]. Moreover, the $CB_{1/2}R$ agonist WIN55212-2 can suppress intestinal activity since it can attenuate the electrically-evoked contractions of the myenteric plexus-longitudinal muscle preparation of the guinea-pig small intestine in a Ca^{2+}-dependent and CB_1R-specific manner [105]. Thus, CB_1R agonists in the in vivo setting can also suppress the gastrointestinal tract motility [104]. Using whole-cell patch-clamp recordings in brainstem slices, Derbenev et al. [106, 107] have shown that activation of presynaptic CB_1Rs in the dorsal

vagal complex inhibits synaptic transmission to the dorsal motor nucleus of the vagus neurons, which may explain suppression of visceral motor responses caused by cannabinoids.

Furthermore, in the central nervous system CB_1R stimulation can result in inhibition of Ca^{2+}-dependent neurotransmitter release from presynaptic nerve terminals which consequently leads to inhibition of neurotransmission [108]. In chemotherapy-induced nausea and vomiting, the CB_1R-mediated antiemetic action of cannabinoids appears to be directly related to presynaptic inhibition of release of emetic neurotransmitters from nerve terminals. **Figure 4** may help to explain the antiemetic action of cannabinoid CB_1R agonists from the Ca^{2+} perspective. Indeed, the adenylyl cyclase/cyclic AMP (cAMP)/protein kinase A (PKA) signal transduction system is a well-established emetic signaling pathway [109]. PKA activation is known to phosphorylate both Ca^{2+} ion channels on plasma membrane and intracellular endoplasmic IP_3Rs, which respectively increase extracellular Ca^{2+} influx and internal Ca^{2+} release from the sarco/endoplasmic reticulum stores [110]. CB_1Rs are known to be Gi/o-protein coupled receptors which mediates inhibition of adenylate cyclase. This inhibition has been proposed to be the fundamental reason for CB_1R agonists attenuating Ca^{2+}-dependent emetic neurotransmitter release which would ultimately reduce postsynaptic neuronal activation in both dorsal vagal complex and gastrointestinal tract [93, 103]. Moreover, dose-dependent inhibitory action of cannabinoid CB_1R agonists on extracellular Ca^{2+} influx via a number of voltage-gated Ca^{2+} channels residing in the cell membrane including N-type, P/Q type and L-type have been demonstrated in multiple experimental systems [111–115]. Additionally, cannabinoid CB_1R agonists also block $5\text{-}HT_3Rs$ in a non-competitive manner and thus prevent extracellular Ca^{2+} influx [115, 116].

Furthermore, CB_1R agonists appear to inhibit the intracellular Ca^{2+} release channels located on the sarco/endoplasmic reticulum membrane, RyRs. Ca^{2+}-induced Ca^{2+} release is a well-established feature of Ca^{2+} signal amplification. During neuronal activation, Ca^{2+}-induced Ca^{2+} release Ca^{2+} signaling involves increased concentration of cytoplasmic Ca^{2+} via extracellular Ca^{2+} influx through voltage-gated Ca^{2+} channels (e.g., LTCCs) present on the cell membrane, which then causes release of stored intracellular Ca^{2+} from the sarco/endoplasmic reticulum into the cytosol through RyRs [117]. In fact RyRs have a wide distribution in the central nervous system including the brainstem [118]. RyRs not only can regulate Ca^{2+} homeostasis, but also other critical brain functions including neurotransmitter release [117]. Increased serum levels of the pro-inflammatory cytokine, tumor necrosis factor alpha (TNF-α), is associated with chemotherapy-evoked vomiting [119]. TNF-α can excite vagal afferent terminals by augmenting Ca^{2+} release from sarco/endoplasmic reticulum stores via sensitization of RyRs which subsequently amplifies neurotransmission in the brainstem [15]. Cannabinoid CB_1R agonists prevent the TNF-α-evoked sensitization of RyRs and therefore attenuate intracellular Ca^{2+} release from the sarco/endoplasmic reticulum stores [15]. Peripheral RyRs also play a critical role in agonist-evoked Ca^{2+} oscillations in gut epithelial cells [120]. Therefore, the ability of CB_1R agonists in preventing both extracellular Ca^{2+} influx as well as intracellular Ca^{2+} release from the sarco/endoplasmic reticulum stores may be the fundamental mechanisms underlying the broad-spectrum antiemetic efficacy of CB_1R cannabinoid agonists.

3.3. Glucocorticoids

Glucocorticoids, used primarily as anti-allergic and anti-inflammatory drugs. They are also effective, either alone or in combination with other antiemetics, for the suppression of nausea

and vomiting. Indeed, dexamethasone (**Figure 2**), one of the clinically used glucocorticoids, is effective in reducing both acute and delayed chemotherapy-induced nausea and vomiting, and when combined with 5-HT_3 or neurokinin NK_1 antagonists, it is utilized in patients receiving high emetogenic chemotherapy [6]. Glucocorticoids' antiemetic effect has been related to its inhibitory effects in the following facets: (i) glucocorticoids control the inflammatory response involved in mediating chemotherapy-induced nausea and vomiting by reducing the production of inflammatory mediators such as cytokines, chemokines, inducible nitric oxide synthase, and increasing the gene transcription of anti-inflammatory proteins [6]; (ii) glucocorticoids can inhibit 5-HT and substance P release, both of which can evoke emesis [6, 121], (iii) glucocorticoids can cross the blood-brain barrier and can exert direct central inhibitory effects on the nucleus tractus solitarius [6], which may be due to a decrease in abnormal elevation of cytosolic Ca^{2+} concentration as well as downstream Ca^{2+} signals and the maintenance of Ca^{2+} homeostasis within the cell [122], (iv) inhibitory actions of glucocorticoid could also be due to increased release of endocannabinoids, anandamide and 2-arachidonoylglycerol, evoked by glucocorticoid administration which will then be followed by subsequent CB_1R activation as well as glucocorticoid facilitation of synaptic γ-aminobutyric acid (GABA) release and suppression of glutamate release [123, 124]. The endocannabinoid system is composed of CBRs, endocannabinoids and the enzymes involved in their synthesis. Anandamide and 2-arachidonoylglycerol are among the well-studied endocannabinoids and endogenous activators of CBRs [125]. The role of CB_1R agonists as antiemetics was discussed in Section 2.2. It has been suggested that dexamethasone may decrease motion sickness through modulation of the endocannabinoid/CB_1 receptor system on the terminals of the nucleus tractus solitarius neurons that project to the output neurons of the DMNV as well as by endocannabinoid/CB_1 receptor system-mediated inhibition of transmitter release from interneurons of the nucleus tractus solitarius [99, 126]. Selective elevation of 2-arachidonoylglycerol by inhibition of its major metabolic enzyme monoacylglycerol lipase, have been shown to suppress lithium chloride evoked vomiting in the house musk shrew (*Suncus murinus*) [127]. However, intraperitoneal administration of the endocannabinoid 2-arachidonoylglycerol can evoke vomiting in the least shrew in a dose-dependent manner probably via its rapid metabolism to arachidonic acid which is also a potent emetogen in this species [128]. Moreover, the cancer chemotherapeutic agent cisplatin can increase 2-arachidonoylglycerol but not anandamide levels in the least shrew brain [129].

4. Perspective in developing new antiemetic candidates

4.1. Antiemetic efficacy of LTCC blockers in the least shrew model of emesis

Nifedipine along with amlodipine, are among the most studied of Ca^{2+} channels blockers, and both belong to the dihydropyridine subgroup of LTCC antagonists. Relative to nifedipine, a fast and short-acting LTCC antagonist with a plasma half-life of 1.2 h, amlodipine is slow and longer acting, more extensively bound to plasma protein, with a larger volume of distribution, more gradual elimination, with a half-life of over 30 h [130–134]. We have evaluated the antiemetic efficacy of both nifedipine and amlodipine (**Figure 2**) by assessing mean emesis frequency and the percentage of shrews vomiting, and demonstrated that both LTCC blockers [45, 46] behave as broad-spectrum antiemetics when delivered systemically against diverse specific emetogens,

including FPL 64176 (10 mg/kg, i.p.), the peripherally-acting and non-selective 5-HT_3R agonist 5-HT (5 mg/kg, i.p.), the peripherally/centrally-acting and more selective 5-HT_3R agonist 2-Methyl-5-HT (5 mg/kg, i.p.), the dopamine D_2R-preferring agonist quinpirole (2 mg/kg, i.p.), the non-selective dopamine D_2R agonist apomorphine (2 mg/kg, i.p.), the nonselective cholinergic agonist pilocarpine (2 mg/kg, i.p.), the M_1-preferring cholinergic agonist McN-A343 (2 mg/kg, i.p.), and the selective neurokinin NK_1R agonist GR73632 (5 mg/kg, i.p.). The vomiting behavior was recorded for 30 min. Our results suggest that both amlodipine and nifedipine act by suppressing the influx of extracellular Ca^{2+}, thereby delay the onset as well as protecting least shrews from vomiting, further supporting our proposed Ca^{2+} hypothesis of emesis. Nifedipine appears to be more potent than amlodipine against vomiting caused by FPL64176, 5-HT, 2-Methyl-5-HT, GR73632, quinpirole and McN-A343. These potency disparities could be explained in terms of their pharmacokinetic and pharmacodynamic differences [130–139].

Unlike the above tested emetogens which can evoke vomiting within minutes of administration, cisplatin (10 mg, i.p.) requires more exposure time (30–45 min) to begin to induce emesis since only its metabolites are emetogenic. The relative efficacy of amlodipine (5 mg/kg., i.p.) in reducing the frequency of cisplatin-evoked early vomiting by 80% compared with the observed lack of antiemetic action of nifedipine up to 20 mg/kg [45, 46], could be explained in terms of positively charged amlodipine associating more slowly with LTCCs, requiring more exposure time not only to reach its sites of action, but also to compensate for its slower receptor binding kinetics, which can lead to a more gradual onset of antagonism [140]. In addition, intracerebroventricular microinjection of another LTCC antagonist, nitrendipine, has been shown to attenuate nicotine-induced vomiting in the cat [141], which further supports the discussed broad-spectrum antiemetic efficacy of nifedipine and amlodipine as observed in the least shrew model. Cisplatin-based chemotherapeutics induce both immediate and delayed vomiting in humans and in vomit-competent animals [16, 142, 143]. In the least shrew, cisplatin (10 mg/kg, i.p.) causes emesis over 40 h with respective peak early- and delayed-phases occurring at 1–2 and 32–34 h post-injection [144]. Amlodipine, due to its unique pharmacokinetics, may offer practical advantages over other calcium antagonists in cisplatin-evoked delayed emesis.

4.2. Potentiation of antiemetic efficacy of 5-HT_3R antagonists when combined with LTCC blockers

In 1996 Hargreaves and co-workers [20] demonstrated that members of all three major classes of LTCC antagonists can prevent the ability of the 5-HT_3 receptor-selective agonist 1-(m-chlorophenyl)-biguanide to increase intracellular Ca^{2+} concentration in cell lines that possess either one or both of these two different Ca^{2+}-ion channels. The latter interaction is not competitive since the binding site for the different classes of LTCC antagonists appear not to be the same as the serotonin 5-HT_3R binding site itself (i.e., the orthosteric site) but instead, is an allosteric site in the 5-HT_3 receptor channel complex. Furthermore, 5-HT release from enterochromaffin cells can be prevented by antagonists of both 5-HT_3Rs and LTCCs [145, 146]. These findings provide possible mechanisms via which antagonists of both LTCCs and 5-HT_3Rs can mutually prevent the biochemical and behavioral effects of their corresponding selective agonists, including the vomiting behavior induced by their corresponding selective agonists FPL64176 and 2-Methyl-5-HT as we reported previously [45]. We have further demonstrated that when non-effective antiemetic doses of their selective antagonists (nifedipine

and palonosetron, respectively) are combined [45], the combination significantly and in an additive manner attenuate both the frequency and the percentage of shrews vomiting in response to either FPL 64176 or 2-Methyl-5-HT. Furthermore, although nifedipine alone up to 20 mg/kg dose failed to protect shrews from acute cisplatin-induced vomiting, its 0.5 mg/kg dose, significantly potentiated the antiemetic efficacy of a non-effective (0.025 mg/kg) as well as a semi-effective (0.5 mg/kg) dose of palonosetron. In another study we also utilized a combination of non-effective doses of amlodipine (0.5 mg/kg or 1 mg/kg) with a non- or semi-effective dose of the 5-HT_3R antagonist palonosetron (0.05 or 0.5 mg/kg) [46]. The combined antiemetic doses produced a similar additive efficacy against vomiting induced by either FPL 64176 or cisplatin. In fact relative to each antagonist alone, the combination was at least 4 times more potent in reducing the vomit frequency and provided more protection against FPL 64176-induced vomiting. The observed additive antiemetic efficacy of a combination of 5-HT_3- (and/or possibly NK_1-) with LTCC-antagonists in the least shrew suggests that such a combination should provide greater emesis protection in cancer patients receiving chemotherapy in a manner similar to that reported between 5-HT_3- and NK_1-receptor antagonists both in the laboratory [144, 147] and in the clinic [148]. Although in our investigation, the mechanism underlying the additive antiemetic efficacy of combined low doses of LTCC antagonists with 5-HT_3R antagonists was not directly studied, the published literature points to their interaction at the signal transduction level involving Ca^{2+} [20, 149, 150].

4.3. Clinical use of LTCC blockers as anti-nausea/antiemetic medication

There are several published clinical case reports that demonstrate Ca^{2+} channel blockers may provide protection against several causes of nausea and vomiting. The LTCC antagonist flunarizine (**Figure 2**) was shown to reduce cyclic vomiting on acute basis in one patient [151] and prophylactically in 8 other patients [152]. Gabapentin is a gamma-aminobutyric acid (GABA) analog and is predominantly used in the clinic for the management of pain [3]. Gabapentin binds to the alpha-2/delta auxiliary subunits of voltage-gated Ca^{2+} channels (VGCCs) (i.e., LTCCs), and exerts inhibitory actions on trafficking and activation kinetics of VGCCs [153] (**Figure 2**). Moreover, several other reports indicate that gabapentin can also be used as a well-tolerated, less-expensive and promising anti-nausea and antiemetic agent in diverse conditions including: postoperative nausea and vomiting [154, 155], moderately or highly emetogenic chemotherapy-induced nausea and vomiting, particularly effective against delayed chemotherapy-induced nausea and vomiting [156], and both acute and delayed nausea induced by chemotherapy [157], as well as hyperemesis gravidarum [158]. When combined with dexamethasone, gabapentin can also significantly reduce the 24-h incidence of postoperative nausea and vomiting [159]. Alpha-2/delta subunits of VGCCs control transmitter release and further facilitate excitatory transmission [153]. Gabapentin's interaction with neuronal alpha-2/delta subunits of VGCCs and subsequent downregulation of neuronal Ca^{2+} signaling in emesis relevant sites, such as the dorsal vagal complex, is postulated to play a critical role in its anti-nausea and anti-vomiting effects [3].

4.4. Intracellular Ca^{2+} release channels: possible targets for suppression of emesis

A functional and physical linkage between LTCCs and RyRs appears to exist and plays an important role in intracellular Ca^{2+} release following voltage-dependent Ca^{2+} entry through LTCCs

during neuronal depolarization to generate a transient increase in cytosolic Ca^{2+} [160–162]. Physical attachment of IP_3Rs to plasma membrane Ca^{2+} influx channels through conformational coupling has also been proposed as one of the mechanisms connecting depletion of internal Ca^{2+} stores with stimulation of extracellular Ca^{2+} influx [163]. For example, Ca^{2+} release from IP_3Rs was shown to couple with extracellular Ca^{2+} influx through LTCCs in non-excitable cells such as Jurkat human T lymphocytes [164] and drosophila S2 cells [165], as well as in excitable cells such as submucosal neurons in the rat distal colon [166]. We have found that 5-HT_3R-mediated vomiting triggered by 5 mg/kg 2-Methyl-5-HT is insensitive to the intracellular Ca^{2+} release channel IP_3R antagonist 2-APB, but in contrast, was dose-dependently suppressed by the RyR antagonist, dantrolene [25]. Furthermore, a combination of the semi-effective doses of amlodipine and dantrolene was more potent than each antagonist being tested alone [25]. Significant reductions (70–85%) in the frequency of Ca^{2+} mobilizer thapsigargin-evoked vomiting (see Section 1.2) were observed when shrews were pretreated with antagonists of either IP_3Rs (2-APB at 1 and 2.5 mg/kg, i.p.)- or RyRs (dantrolene at 2.5 and 5 mg/kg, i.p.)-ER luminal Ca^{2+} release channels. Moreover, while a mixture of 2-APB (1 mg/kg) and dantrolene (2.5 mg/kg) did not offer additional protection than what was afforded when each drug administered alone, a combination of the latter doses of 2-APB plus dantrolene with a partially effective dose of nifedipine (2.5 mg/kg), led to a complete elimination of thapsigargin-evoked vomiting [70]. In another set of experiments [167], we found that pretreatment with the IP_3R inhibitor 2-APB causes a significant reduction in NK_1R agonist GR73632-induced emesis, however the RyR inhibitor dantrolene did not. Thus, RyRs and IP_3Rs can be differentially modulated by various emetogens and their antagonists provide further efficacy when combined with LTCC antagonists (**Figure 2**). Suppression of Ca^{2+} release from the sarco/endoplasmic reticulum stores through IP_3Rs and RyRs may be additional targets for the prevention of nausea and vomiting.

4.5. Ca^{2+}-related signaling pathways in emesis

4.5.1. The role of cAMP-PKA in vomiting

In mammals, cyclic AMP (cAMP) is synthesized by 10 adenylate cyclase isoforms [168]. One of the best-studied second messenger molecules downstream of selected G-protein coupled receptors is cAMP. It is an example of a transient and diffusible second messenger involved in signal propagation by integrating multiple intracellular signaling pathways [169]. cAMP activates protein kinase A (PKA) which results in phosphorylation of downstream intracellular signals. The adenylyl cyclase/cAMP/PKA signaling pathway can phosphorylate Ca^{2+} ion-channels found on the plasma membrane and intracellular IP_3Rs [110]. These Ca^{2+} channels respectively increase extracellular Ca^{2+}-influx and intracellular Ca^{2+}-release [110]. The emetic role of cAMP has been well established (**Figure 5**), since microinjection of cAMP analogs (e.g., 8-bromocAMP) or forskolin (to enhance endogenous levels of cAMP) in the brainstem dorsal vagal complex emetic locus area postrema, not only can increase electrical activity of local neurons, but also induces vomiting in dogs [170]. Moreover, administration of 8-chlorocAMP as a potential chemotherapeutic in cancer patients can evoke nausea and vomiting [171]. Furthermore, phosphodiesterase inhibitors (PDEI) such as rolipram prevent cAMP metabolism and consequently increase cAMP tissue levels, which leads to excessive nausea and vomiting in humans [172]. In fact, one major side-effect of older PDEIs is excessive nausea and vomiting which often precludes their use in the clinical setting [173]. In addition, we have

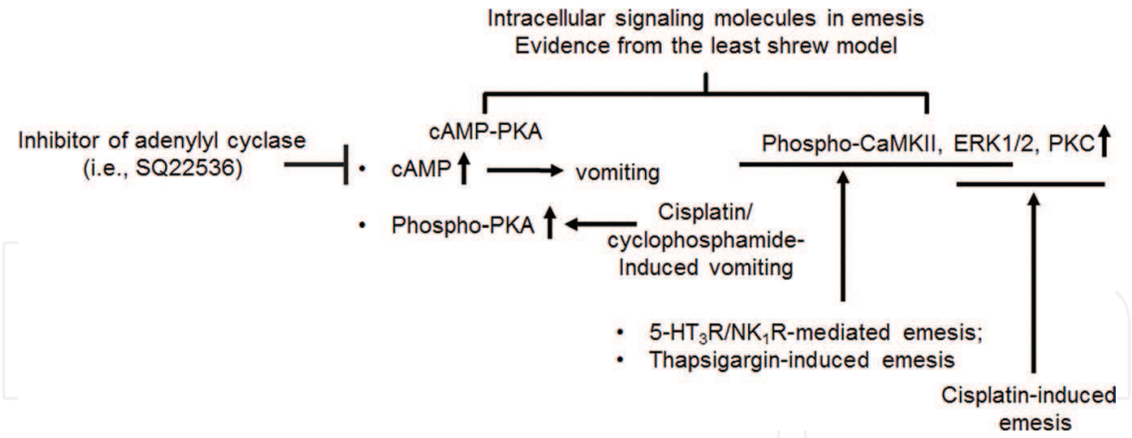

Figure 5. Summarized behavioral and biochemical evidence for intracellular signaling molecules (cAMP, PKA, CaMKII, ERK1/2, PKC) related to emesis based on the least shrew emesis model. First, cyclic AMP (cAMP) is synthesized by adenylate cyclase and cAMP activates protein kinase A (PKA) [110, 168]. The adenylyl cyclase/cAMP/PKA signaling pathway can mediate vomiting. Indeed, increased levels of endogenous cAMP can evoke vomiting in animal models [109, 170] as well as humans [171–173], which can be prevented by adenylate cyclase inhibitor SQ22536 [109]. Evoked PKA-phosphorylation is associated with peak vomit frequency during both immediate- and delayed-phases of vomiting caused by cancer chemotherapeutics including cisplatin and cyclophosphamide in the least shrew [109, 144, 149]. In addition, Ca^{2+}/calmodulin kinase IIα (CaMKIIα) and extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) phosphorylation in the least shrew brainstem were elevated in vomiting evoked by the 5-HT_3R agonist 2-Methyl-5-HT [46], thapsigargin [70], or the selective NK_1R agonist GR73632 [167]. Phosphorylation of protein kinase Cα/βII (PKCα/βII) and ERK1/2 in least shrew brainstem were also upregulated in the vomiting induced by cisplatin [144, 149].

demonstrated that increased brain cAMP levels evoke vomiting which can be prevented by SQ22536 (**Figure 5**), an inhibitor of adenylyl cyclase [109]. Moreover, PKA-phosphorylation is associated with peak vomit frequency during both immediate- and delayed-phases of vomiting caused by either cisplatin or cyclophosphamide in the least shrew [109, 144, 149] (**Figure 5**).

4.5.2. Activation and inhibition of CaMKII, ERK1/2, PKC, and Akt are correspondingly linked to emesis induction and prevention

Vomit-associated Ca^{2+} mobilization as well as time-dependent Ca^{2+}/calmodulin kinase IIα (CaMKIIα) and extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) phosphorylation in the least shrew brainstem occurs: (i) following 5-HT_3R-evoked vomiting caused by its selective agonist 2-Methyl-5-HT [46], (ii) thapsigargin-induced emesis in the least shrew [70], as well as (iii) NK_1R-mediated vomiting evoked by the selective NK_1R agonist GR73632 in the least shrew [167] (**Figure 5**). Our additional behavioral evidence that inhibitors of CaMKII or ERK1/2 attenuate the evoked emesis provides further credence for involvement of CaMKII and ERK1/2 downstream of the discussed emetic receptors/effectors. Furthermore, other published evidence support phosphorylation of protein kinase Cα/βII (PKCα/βII) and ERK1/2 in least shrew brainstem are associated with cisplatin-induced emesis [144, 149] (**Figure 5**). In fact significant upregulation of ERK1/2 phosphorylation occurs with peak vomit frequency during both the immediate and delayed phases of emesis caused by cisplatin in the least shrew [144, 149].

It has been suggested that glucocorticoids' antiemetic efficacy could be due to their anti-inflammatory effects [174] probably via a reduction in the synthesis of prostaglandins and leukotrienes [175], both of which can be increased during chemotherapy [6]. Although not all, but several prostaglandins (e.g., PGE2 and PGF2a) and cysteinyl leukotrienes (e.g., LTC_4

and LTD_4), appear to be potent emetogens [72, 149, 176, 177]. Our findings demonstrate that unlike other leukotrienes (e.g., LTA_4, LTB_4 and LTF_4), the above discussed leukotrienes are effective emetogens with the following potency order: $LTC_4 = LTD_4 > LTE_4$. Regarding LTC4, the evoked vomiting was shown to be suppressed in a dose-dependent manner in the least shrew by the antiasthmatic drug pranlukast, the corresponding cysteinyl leukotrienes receptor 1 (CysLT1R) antagonist [72]. Although not available in the USA, the cost of other members of this class of drugs (montelukast and Zafirlukast) that are sold in the USA is less than one dollar per pill. Based on pranlukast's efficacy against LTC_4-induced vomiting [72], we envisaged it may have potential utility against cisplatin-evoked emesis. Our most recent publication [178] shows the potential of pranlukast (currently used for the treatment of various respiratory disorders including asthma), as a new class of antiemetic for the suppression of the acute- and delayed- phases of cisplatin-evoked vomiting in the least shrew. An intraperitoneal (i.p.) dose of 10 mg/kg pranlukast by itself significantly reduced the mean frequency of vomits by 70% and fully protected 46% of least shrews during the delayed-phase of cisplatin (10 mg/kg, i.p.)-evoked vomiting. Although pranlukast tended to substantially reduce both the mean frequency of vomits and the number of shrews vomiting during the early-phase, these reductions failed to attain significance. When pranlukast was combined with a first (tropisetron)- or a second (palonosetron)-generation 5-HT_3R antagonist, it potentiated their antiemetic efficacy during both acute- and delayed-phases of cisplatin-evoked vomiting. Moreover, pranlukast potentiated the antiemetic efficacy of serotonin 5-HT_3 receptor antagonists, tropisetron and palonosetron, against chemotherapy-induced nausea and vomiting. In fact per hour efficacy antiemetic profile of pranlukast combined with palonosetron or tropisetron during both phases of chemotherapy-induced nausea and vomiting in the least shrew resembles those of: (i) the NK_1 receptor antagonist netupitant (5 mg/kg) plus palonosetron (0.1 mg/kg) in the same species [144]; (ii) netupitant plus ondansetron in ferrets [179]; and (iii) ondansetron plus aprepitant in combination with dexamethasone in ferrets [179]; and (iv) palonosetron plus netupitant in combination with dexamethasone in ferrets [179]. If analogs of pranlukast such as montelukast and zafirlukast can also provide similar antiemetic potential, then clinical trials should be initiated since this class of drugs are relatively inexpensive than available effective antiemetic regimens against chemotherapy-induced nausea and vomiting. Our related biochemical data indicates the mechanisms of antiemetic action of pranlukast are linked to suppression of cisplatin-elicited PKCα/βII, ERK1/2 and PKA activation (phosphorylation) in the least shrew brainstem [178]. Moreover, suppression of these signaling molecules may be shared in the anti-inflammatory signaling pathway of pranlukast.

When antiemetic mechanism of action of pranlukast against LTC4-induced vomiting or cisplatin-induced responses is discussed, Ca^{2+} is also an essential element. Montelukast and pranlukast were found to inhibit nucleotide-induced Ca^{2+} mobilization in a human monocyte-macrophage-like cell line, DMSO-differentiated U937 [180]. CysLT1 receptors belonging to the rhodopsin family of the G protein-coupled receptor genes respond to LTD4 with a strong increase in cytosolic Ca^{2+} concentration partially sensitive to pertussis toxin, and with the activation of the Ras-MAPK cascade totally dependent upon $G_{i/o}$ [144]. These signaling effects were totally inhibited by various specific CysLT1-receptor antagonists, and CysLT1 antagonists inhibit both the P2Y agonist-induced activation of phospholipase C and intracellular Ca^{2+} mobilization [144].

5. Conclusion

Chemotherapy-induced nausea and vomiting is a particularly distressing side-effect of chemotherapeutics for oncology patients both physically and psychologically. The use of 5-HT_3R antagonists combined with NK_1R antagonists, has enhanced physician's ability to further suppress nausea, the rates of acute- and delayed-vomiting in cancer patients receiving chemotherapy. In addition to the commonly reported adverse effects of these agents (including headache, diarrhea, constipation, hiccups, and fatigue), many patients still experience nausea and delayed vomiting [181–183]. Furthermore, the use of second generation 5-HT_3R and NK_1R antagonists for the prevention of chemotherapy-induced nausea and vomiting is currently cost-prohibitive for most patients in the world. Mechanisms that cause nausea are only partially understood and probably in part overlap with those of vomiting. There are still unmet needs for newer and less expensive therapeutic options to improve the treatment across the entire spectrum of chemotherapy-induced nausea and vomiting. Additional studies should involve combinations of agents that inhibit other neurotransmitter systems involved in nausea and vomiting.

As concluded in **Figure 2**, this systematic review shows clear evidence that Ca^{2+} modulation is an important contributor to antiemetic and probably anti-nausea signaling pathways. LTCC blockers, antagonists of intracellular IP_3Rs and RyRs Ca^{2+} release channels as well as CysLT1R antagonists have the potential to provide less expensive (e.g., nifedipine, amlodipine, dantrolene, and pranlukast) broad-spectrum antiemetic agents for the clinic against diverse causes of nausea and vomiting. The discussed findings from the least shrew should help to open new avenues of research in other established animal models of emesis as well as in patients, targeting not only the already discussed Ca^{2+} channels, but also other Ca^{2+} channels that exist on both the plasma membrane and the membranes of intracellular organs such as the sarco/endoplasmic reticulum and mitochondria.

Acknowledgements

This work was supported by NIH/NCI grant (RO1CA207287) and Western University of Health Science startup fund to NAD.

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