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Intracellular Vomit Signals and Cascades Downstream of Emetic Receptors: Evidence from the Least Shrew (*Cryptotis parva*) Model of Vomiting

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Abstract

Nausea and vomiting are often considered as stressful symptoms of many diseases and drugs. In fact they are the most feared and debilitating side-effects of many cancer chemotherapeutics and the main cause of patient noncompliance. Despite years of substantial research, the intracellular emetic signals are at best poorly understood or remain unknown. Among different receptor-mediated emetic signaling cascades, one potential converging signal appears to be changes in the cytosolic concentration of $Ca2^{+}$. In this editorial, we focus on $Ca2^{+}$-related intracellular signals underlying emesis mediated by various emetogens. This strategy will help us understand common signaling mechanisms downstream of diverse emetogens and should therefore promote development of new antiemetics for the treatment nausea and vomiting caused by diverse diseases, drugs, as well as viruses and bacterial infections.

Keywords: Emetogens; Nausea; Intracellular emetic signals

Introduction

Nausea and vomiting (emesis) can be both a reason and/or symptoms of diseases, drugs (e.g. chemotherapeutics [1-3], opiates [4]), conditions (pregnancy [5], motion sickness [6], food poisoning [7]), as well as bacterial [8] and viral infections [9]. Treatment of these symptoms require millions of patient visits per year to the doctors' office or hospitals in the USA [10,11]. These symptoms are an important gastrointestinal problem which worsens the both quality of patient life and treatment. So it is noteworthy to explain and investigate mechanisms. Although anti-emetics can be effective against certain types of vomiting, oftentimes they do not provide complete protection and frequently lack broad-spectrum antiemetic efficacy. While nausea and vomiting are often considered as stressful symptoms of many diseases and drugs, they are the most feared and debilitating side-effects of cancer chemotherapeutics (e.g. cisplatin) in patients and the main cause of patient noncompliance [2,3]. Moreover, the cost of treatment of nausea and vomiting are considered economic burden to the healthcare service not only in the USA, but also in the world at large [10,11].

The role of different cell membrane-bound emetic receptors in vomiting is fairly well understood [1]. However, despite continued pre-clinical research, their corresponding downstream intracellular biochemical emetic mediators are at best poorly defined or remain unknown [1]. Of critical importance is that major knowledge gaps exist in the emesis field since not only limited information is available regarding intracellular emetic signals activated by diverse emetogens, but also virtually no evidence exist on potential point(s) of signal convergence (e.g. Ca^{2+}) among different receptor-mediated emetic signaling cascades. One potential converging signal in vomiting appears to be changes in cytosolic Ca^{2+} concentration [12]. Extracellular Ca^{2+} gaining access inside cells can serve as a second messenger to initiate cellular events such as protein phosphorylation [13], neurotransmitter release [14] and Ca^{2+} influx [15]. We have recently provided an overview of the involvement Ca^{2+} mobilization in the process of vomiting evoked by diverse emetogens [12]. First, both selective emetogens that activate specific emetic receptors (such as tachykininergic NK_1 [16], serotonergic 5-HT_3 [17], dopaminergic D_2 [18]), as well as nonspecific emetogens (e.g. cisplatin), can evoke intracellular Ca^{2+} rise and subsequently initiate downstream Ca^{2+} activated emetic signals. Second, cisplatin, one of the oldest and most widely used cancer chemotherapeutics [19], induces nausea and vomiting via Ca^{2+}-dependent release of multiple neurotransmitters (serotonin (5-HT), substance P (SP), dopamine, etc.) from central emetic loci in the dorsal vagal complex (DVC) of the

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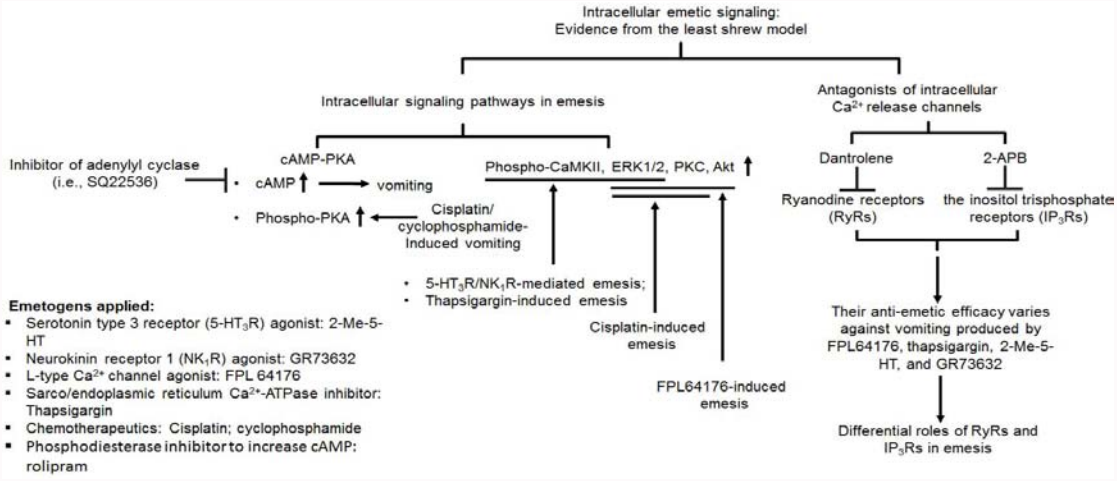

Figure 1: Summarized behavioural and biochemical evidence for intracellular emetic signals based on the least shrew emesis model.

brainstem [1,20]. The DVC contains the emetic nuclei nucleus tractus solitarius (NTS), the dorsal motor nucleus of the vagus (DMNX) and the area postrema (AP). Not only activation of these emetic nuclei can directly evoke vomiting, but also they may interact with peripheral emetic loci including the enteric nervous system (ENS) and enterochromaffin cells (EC cells) of the gastrointestinal tract (GIT) via afferent/efferent vagal nerves in the process of chemotherapy-induced nausea and vomiting (CINV) [1].

In fact cisplatin-like chemotherapeutics can evoke early (day 1) and delayed (days 3-7) vomiting in almost all patients [21]. The neurotransmitter basis of CINV suggests that 5-HT and SP are concurrently released from EC cells of the GIT and brainstem emetic loci during both phases of vomiting [1,22]. 5-HT plays the dominant and SP a smaller role in the early phase. Indeed, both neurotransmitters are concomitantly released from the EC cells to stimulate their corresponding serotonin 5-HT_3 receptors (5-HT_3Rs) and SP neurokinin (tachykinin) NK_1 receptors (NK_1Rs) located onthe GIT vagal afferent neurons leading to afferent emetic signaling to the brainstem. Subsequently, the brainstem emetic nuclei are activated, which evoke vomiting via vagal efferents [1]. The delayed phase is largely a consequence of activation of central NK_1Rs subsequent to SP release in the medial NTS [23] and to a lesser extent due to release of both SP and 5-HT in the GIT [1]. The acute and delayed phases of CINV causes distressing effects which affect the well-being and quality of life of cancer patients receiving cisplatin-like chemotherapeutics [24]. The current clinically-preferred prophylactic antiemetic regimen against CINV includes the 5-HT_3R antagonist palonosetron combined with the NK_1R antagonist netupitant, as well as the corticosteroid dexamethasone for its anti-inflammatory effects [10].

Unlike primates, only some animal species are vomit competent. The common animal models of vomiting currently used in the laboratory include ferrets, house musk shrews (*Suncus murinus*) and least shrews (*Cryptotis parva*) [1]. The least shrew is a vomit-competent mammal whose reactions to common emetogens have been well defined and correlate closely with human responses [1]. In this review, we mainly discuss intracellular signal transduction systems involved in emesis evoked by diverse agents including emetic receptor agonists as well as cisplatin in the least shrew model, and highlight evidence for development of potential therapeutics for control of vomiting.

The involvement of intracellular Ca^{2+} release channels in emesis

Ca^{2+} induced Ca^{2+} release, refers to the process of extracellular Ca^{2+} influx via activation of voltage-operated Ca^{2+} channels in the cell membrane which subsequently mobilizes intracellular Ca^{2+} release from the sarcoplasmic/endoplasmic reticulum (SER) Ca^{2+} stores, resulting in a transient increase in the cytosolic concentration of Ca^{2+} [25,26]. Intracellular Ca^{2+} release from the SER into cytoplasm is mediated by inositol trisphosphate receptors (IP_3Rs) and ryanodine receptors (RyRs) found in the SER membrane [27].

The selective L-type Ca^{2+} channel (LTCC) agonist FPL64176 is an intracellular Ca^{2+} mobilizing agent and causes vomiting in all tested least shrews at a 10 mg/kg intraperitoneal (i.p.) dose [28,29]. Recently we explored the role of RyRs and IP_3Rs in intracellular Ca^{2+} release following FPL64176 evoked vomiting through pharmacological use of their respective inhibitors, dantrolene and 2-APB. We found FPL64176 induced emesis was insensitive to 2-APB, but in contrast, both the frequency and percentage of shrews vomiting were dose-dependently suppressed by dantrolene. Similar to FPL64176 mediated vomiting, we have shown that the 5-HT_3R-mediated vomiting was insensitive to 2-APB, but in contrast, both emetic parameters were dose-dependently suppressed by dantrolene [30].

The intracellular Ca^{2+} mobilizing agent thapsigargin, is a selective $SERCa^{2+}$ ATPase (SERCA) inhibitor, which increases cytosolic Ca^{2+} concentration via an initial intracellular Ca^{2+} store depletion followed by extracellular Ca^{2+} entry [31-33]. In contrast with FPL64176 evoked vomiting, pre treatment with either dantrolene or 2-APB, led to significant reductions in the frequency of thapsigargin-induced vomiting [34]. We therefore concluded that both Ca^{2+} channels (RyRs and IP_3Rs) are involved in thapsigargin-induced vomiting. In another set of experiments [35], 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, we suggest that RyRs and IP_3Rs can be differentially modulated by various emetogens (Figure 1), and suppression of Ca^{2+} release from SER-stores through IP_3Rs and RyRs may be additional targets for the prevention of nausea and vomiting.

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

The role of cAMP-PKA in vomiting: In mammals cyclic AMP (cAMP) is synthesized by 10 adenylate cyclase isoforms [36]. One of the best-studied second messenger molecules downstream of selected G-protein coupled receptors is cAMP. It is an example for a transient and diffusible second messenger which is involved in signal propagation by integrating multiple intracellular signaling pathways [37]. 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 [38]. These Ca^{2+} channels respectively increase extracellular Ca^{2+} influx and intracellular Ca^{2+} release [38]. The emetic role of cAMP has been well established, since microinjection of cAMP analogs (e.g. 8-bromocAMP) or forskolin (to enhance endogenous levels of cAMP) in the brainstem DVC emetic locus area postrema, not only can increase electrical activity of local neurons, but also induces vomiting in dogs [39]. Moreover, administration of 8 chloro cAMP in cancer patients can evoke nausea and vomiting [40]. 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 [41]. In fact one major side-effect of older PDEIs is excessive nausea and vomiting which often precludes their use in the clinical setting [42]. In addition, we have demonstrated that increased brain cAMP levels-induced vomiting can be prevented by SQ22536, an inhibitor of adenylyl cyclase [43] as well as 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 [43-45].

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 [30], ii) thapsigargin-induced emesis in the least shrew [34], as well as iii) SP tachykinin NK_1R-mediated vomiting evoked by the selective NK_1R agonist GR73632 in the least shrew [35]. 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. In addition, other published evidence demonstrate that phosphorylation of protein kinase Cα/βII (PKCα/βII) and ERK1/2 in least shrew brainstem are associated with cisplatin-induced emesis [44,45]. 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 [44,45]. Our most recent publication 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. Our related biochemical data indicates the mechanisms of antiemetic action of pranlukastare linked to suppression of cisplatin-elicited PKCα/βII, ERK1/2 and PKA activation (phosphorylation) in the least shrew brainstem [46].

Our other findings (unpublished data) from the least shrew reveal that phosphorylation of PKCα/βII, CaMKIIα, ERK1/2 and protein kinase B (Akt) contribute to FPL64176 mediated vomiting and are under regulation of Ca^{2+} mobilization which acts as one of the earliest and requisite events in the signal transduction pathways underlying emesis [12]. Indeed, FPL64176 exposure increased phosphorylation of these intracellular emetic signals in the brainstem in a time-dependent manner. In addition, in the presence of inhibitors of PKC (GF109203X), CaMKII (KN93), or ERK1/2 (U0126), both the frequency and percentage of shrews vomiting in response to FPL64176 were decreased. To evaluate the significance of Akt phosphorylation in FPL64176-induced vomiting, we also determined the anti-emetic effect of LY294002, an inhibitor of its upstream enzyme phosphatidylinositol-3 kinase (PI3K). Our results revealed that phosphorylation of Akt also contributes to FPL64176-evoked vomiting (unpublished data).

Conclusion

In both the periphery and the brainstem, emetic neurotransmitters/mediators may act independently or in combination to evoke vomiting. With the results reviewed in this editorial, multifaceted comprehensive investigations are required to ascertain the "cross talks of intracellular emetic signaling" among diverse specific and nonspecific emetogens including cisplatin. Although antiemetics may be clinically effective against some causes of vomiting, oftentimes they fail to provide complete protection and furthermore most lack broad-spectrum antiemetic efficacy [1]. Therefore, there are still unmet needs for broader and less expensive therapeutic options to improve antiemetic clinical efficacy. Additional studies should involve potential antiemetic compounds targeting common or specific intracellular emetic signaling pathways, alone or in combination with conventional drugs of choice.

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