Role of Calcium in Vomiting: Revelations from the Least Shrew Model of Emesis

Nissar A. Darmani and Weixia Zhong

Department of Basic Medical Sciences, College of Osteopathic Medicine of the Pacific, Western University of Health Sciences, Pomona, CA 91766, USA

ABSTRACT

Cisplatin-like chemotherapeutics cause vomiting via release of multiple neurotransmitters (dopamine, serotonin, or substance P) from the gastrointestinal enterochromaffin cells and/or the brainstem via a Calcium (Ca^{2+}) dependent process. In addition, evidence from literature indicate that Ca^{2+} signaling is also triggered subsequent to activation of other emetogenic receptors including serotonergic 5-HT_{3}, tachykinin NK_{1}, dopamine D_{2}, and histaminergic H_{1} receptors. Moreover, other emetogens such as prostaglandins, cisplatin, rotavirus NSP4 protein and bacterial toxins have the ability to induce intracellular Ca^{2+} elevation. Our findings demonstrate that application of the L-type Ca^{2+} channel (LTCC) agonist FPL-64176 or the Ca^{2+} mobilizing agent thapsigargin (a sarco/endoplasmic reticulum Ca^{2+-ATPase inhibitor) cause vomiting in the least shrew, whereas blockade of LTCC by corresponding antagonists (nifedipine or amlodipine) not only provide broad-spectrum antiemetic activity against diverse emetogens including agonists of 5-HT_{3}, NK_{1}, D_{2} and M_{1} receptors, but can also potentiate the antiemetic efficacy of well-established antiemetic palonosetron against the non-specific emetogen, cisplatin. The transmission of emesis signals in the gastrointestinal tract and brainstem is crucially dependent on Ca^{2+} channels in neurons. In this review, we will examine the current knowledge on the role of Ca^{2+} channels and Ca^{2+}-dependent signaling pathways in the perception and modulation of emesis.

KEYWORDS: Calcium; Cisplatin; 5-HT_{3}, receptor; L-type Ca^{2+} channel; Ryanodine receptor; Signaling pathway.

ABBREVIATIONS: LTCC: L-type Ca^{2+} channel; GIT: Gastrointestinal tract; DVC: Dorsal Vagal Complex; DMNX: Dorsal Motor Nucleus of the Vagus; AP: Area Postrema; NTS: Nucleus Tractus Solitarius; ER: Endoplasmic Reticulum; VOCs: Voltage-Operated Channels; ROCs: Receptor-Operated Channels; SMOCs: Second Messenger-Operated Channels; SOCs: Store-Operated Channels; EC: Enterochromaffin; SERCA: Sarcoplasmic/Endoplasmic Reticulum Ca^{2+-ATPase; SER: Sarcoplasmic/Endoplasmic Reticulum; IP_{3}R: Inositol Trisphosphate Receptors; RyRs: Ryanodine Receptors; TRPC: Transient Receptor Potential Channels; STIM1: Stromal interacting molecule 1; CICR: Calcium-Induced Calcium-Release; PKA: Protein Kinase A.

CALCIUM HYPOTHESIS OF EMESIS

Many neurotransmitters/drugs have been implicated in the induction of vomiting including dopamine, acetylcholine, histamine, opiates, serotonin (5-HT), substance P (SP), prostaglandins and leukotrienes, to name a few. Chemotherapeutics such as cisplatin induce vomiting via the release of a number of the above-discussed neurotransmitters/mediators in both the Gastrointestinal tract (GIT) and the brainstem Dorsal Vagal Complex (DVC) emetic nuclei including the Nucleus Tractus Solitarius (NTS), the Dorsal Motor Nucleus of the Vagus (DMNX) and the Area Postrema (AP). Calcium (Ca^{2+}) is one of the simplest yet most dynamic signaling ions poised at the center of a complex network of signal transduction pathways whose
integration controls cellular pathophysiology. At rest, diverse cells have strict and well-regulated mechanisms to maintain low nM cytosolic Ca$^{2+}$ levels. However, in response to synaptic activity, cytosolic Ca$^{2+}$ can be elevated up to 5 µM. Thus, agonists can increase cytosolic Ca$^{2+}$ levels via both mobilization of intracellular stores (e.g. Endoplasmic Reticulum=ER) and influx from extracellular fluid. The NK₁ receptor is G-protein coupled and can increase cytosolic Ca$^{2+}$ concentration via extracellular influx. In addition, the 5-HT₃ receptor is a Ca$^{2+}$-permeable ligand-gated ion channel. 5-HT$_{3}$ receptor can evoke membrane depolarization which consequently increases cytoplasmic Ca$^{2+}$ levels via extracellular influx through L-type- and 5-HT$_{3}$-receptor Ca$^{2+}$-permeable channels. Other emetogens such as agonists of dopamine D$_{2}$, cholinergic M$_{3}$, histaminergic H$_{1}$ and opiate u$_{16,17}$-receptors, as well as cisplatin, prostaglandins, rotavirus NSP4 protein$^{21,22}$ and bacterial toxins$^{23,24}$ have also the potential to induce extracellular Ca$^{2+}$ influx. Therefore Ca$^{2+}$ mobilization can be an important aspect of emesis induction since it is involved in triggering neurotransmitter release, coupled with receptor activation and excitation-transcription coupling.$^{25}$

**L-TYPE CA$^{2+}$ CHANNELS AND EMESIS**

**Emetic Potential of L-type Ca$^{2+}$ Channel Agonists**

A variety of Ca$^{2+}$-permeable ion-channels are present in the plasma membrane, which allow extracellular Ca$^{2+}$ influx into the cell. These include Voltage-Operated Channels (VOCs), Receptor-Operated Channels (ROCs), Second Messenger-Operated Channels (SMOCs) and Store-Operated Channels (SOCs). Voltage-gated Ca$^{2+}$ channels can be divided into L-type, P/Q-type, N-type, R-type, and T-type.$^{25}$ Voltage-gated L-type Ca$^{2+}$ channels are activated by membrane depolarization, and serve as the principal route of Ca$^{2+}$ entry in electrically excitable cells such as neurons and muscle.$^{25}$ Our study$^{30}$ provided the first evidence that the opening of plasma membrane LTCCs by the corresponding selective agonist FPL-64176$^{29}$ produces robust vomiting both in terms of its frequency and the percentage of animals vomiting. All tested shrews vomited at the 10 mg/kg dose of FPL 64176 administered intraperitoneally (i.p.).

**Antiemetic Potential of LTCC Blockers**

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 short-acting LTCC antagonist; amlodipine is much longer acting, with a larger volume of distribution and more gradual elimination.$^{30,32}$ We have evaluated the broad-spectrum antiemetic potential of nifedipine$^{28}$ and amlodipine$^{33}$ against diverse specific (e.g. receptor selective or non-selective agonists) and non-specific (e.g. cisplatin) emetogens. Both nifedipine and amlodipine exhibited broad-spectrum antiemetic activity against diverse emetogens, however, their potency and efficacy differed substantially (Table 1). More specifically, amlodipine pretreatment significantly attenuated both the frequency and percentage of shrews vomiting in response to:

i. FPL-64176 (10 mg/kg, i.p.) in a dose-dependent manner, and provided complete protection at 5-10 mg/kg. In comparison, nifedipine reduced these emetic parameters with ID$_{50}$ values 3.5 to 6.4 times lower. Precisely, pretreatment with nifedipine significantly attenuated the frequency and percentage of FPL-64176-induced vomiting in a dose-dependent manner with significant reductions occurring at its 0.5, 2.5 and 5 mg/kg doses. Thus, FPL-64176-induced emesis appears to be more sensitive to nifedipine.

ii. The peripherally-acting and non-selective 5-HT$_{3}$ receptor agonist 5-HT (5 mg/kg, i.p.) with substantial protection at 5 and complete protection at 10 mg/kg. Likewise, nifedipine pretreatment (1 and 2.5 mg/kg) blocked emesis caused by 5-HT in a dose-dependent but more potent manner with significant suppression in both the frequency and percentage of shrews vomiting at its 2.5 mg/kg. In addition, amlodipine in a dose-profile similar to that of nifedipine, suppressed both the frequency and percentage of shrews vomiting caused by the peripherally/centrally-acting and more selective 5-HT$_{3}$R agonist 2-Me-5-HT (5 mg/kg, i.p.) with respective ID$_{50}$ values 2-12 times larger than that of nifedipine.$^{28,33}$ Thus, comparatively nifedipine appears to be more potent than amlodipine in suppression of emetic behaviors evoked by 2-Me-5-HT.

iii. The dopamine D$_{2}$ receptor-preferring agonist quinpirole (2 mg/kg, i.p.). However, amlodipine only managed to significantly suppress the frequency of the induced vomiting by 80-90% in 40-50% of tested shrews with respective ID$_{50}$ values 20-24 times larger than that of nifedipine. Moreover, while nifedipine totally protected shrews from quinpirole (2 mg/kg)-induced emesis at 1 mg/kg, amlodipine had no such effect even at larger doses. Unexpectedly, both antiemetics, in a similar dose-range, suppressed both the frequency and percentage of shrews vomiting in response to the non-selective dopamine D$_{2}$ receptor agonist apomorphine (2 mg/kg, i.p.) with identical ID$_{50}$ values (Table 1).

iv. The non-selective cholinergic agonist pilocarpine (2 mg/kg, i.p.) with respective ID$_{50}$ values between 2 and 4.6 mg/kg, whereas nifedipine lacked such efficacy. On the contrary, both amlodipine and nifedipine dose-dependently suppressed the described emetic parameters in response to administration of the M$_{3}$-preferring cholinergic agonist, McN-A343 (2 mg/kg, i.p.), nifedipine being 5 times more potent with complete vomit protection achieved at the 5 mg/kg dose (Table 1).

v. The selective tachykinin NK$_{1}$ receptor agonist GR73632 (5 mg/kg, i.p.). However, the vomit frequency was reduced by 90% at the 10 mg/kg dose of amlodipine, and complete protection was only afforded in 50% of shrews at this dose. Nifedipine not only appears to be 7-12 times more potent than amlodipine in reducing the GR73632-induced emetic parameters by 50%, but also provides complete protection at 5 mg/kg.
Thus, nifedipine appears to be 2-24 times more potent than amlovidpine against vomiting caused by FPL 64176, 5-HT, 2-Me-5-HT, GR73632, quinpirole and McN-A-343. These potency disparities could be explained in terms of their pharmacokinetic and pharmacodynamic differences. In fact nifedipine has a rapid onset of action and reaches peak plasma concentration within 30 min of administration with a short duration of action (half-life=1-2 h).34,35 On the other hand, amlodipine has a long duration of action (half life=8-35 h) and reaches peak plasma concentration between 6 and 8 hour with a slow onset of action.36,37 Since both antiemetics were administered 30 min prior to the administration of the discussed emetogens, it is likely that amlovidpine may not have had sufficient time to reach its sites of action, thus having lower potency. In addition, the positively charged amlovidpine associates more slowly with the l-type Ca2+ channel, which can lead to a more gradual onset of antagonism.38

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 in the least shrew since only its metabolites are emetogenic.39 Lack of antiemetic action of nifedipine versus the efficacy of amlovidpine in reducing the frequency of cisplatin-induced vomiting by 80%28,33 could be explained in terms of amlovidpine having more exposure time not only to reach its sites of action, but also to compensate for its slower receptor binding kinetics. Another potential contributing factor for the efficacy of amlovidpine against cisplatin-induced vomiting is its ability to bind an additional Ca2+ site.31

The discussed broad-spectrum antiemetic efficacy of nifedipine and amlovidpine in the least shrew is further supported by scant available clinical case reports in which the LTCC antagonist flunarizine was shown to reduce cyclic vomiting on acute basis in one patient40 and prophylactically in 8 other patients.41 In addition, intracerebroventricular microinjection of nitrendipine has been shown to attenuate nicotine-induced vomiting in the cat.42 More importantly, LTCCs appear to attenuate blood pressure to normal basal levels in hypertensive animals and patients, but do not affect the blood pressure of normotensive animals and patients.42,43 Thus, the broad-spectrum antiemetic potential of both nifedipine and amlovidpine against the diverse selective and non-selective emetogens in the least shrew further supports our proposed Ca2+ hypothesis and warrants initiation of clinical trials for determination of clinically-useful LTCC antagonist antiemetics.

**CROSS-TALK BETWEEN LTCCS AND 5HT3RS**

Recently we have found that the second generation 5-HT3 receptor antagonist palonosetron (Rojas and Slusher, 2012), can suppress the ability of FPL 64176 to cause vomiting in the least shrew in a dose-dependent and potent manner.28 Indeed, complete blockade of 2-Me-5-HT-induced vomiting was achieved at 10 mg/kg dose of nifedipine, whereas a 10 mg/kg dose of potent and selective 5-HT3 receptor antagonists such as tropisetron,47 or palonosetron, could not provide such complete protection against 2-Me-5-HT-induced vomiting in least shrews under similar experimental conditions.28 These findings suggest that FPL 64176, 2-Me-5-HT, or serotonin, probably drive extracellular Ca2+ through both L-type- and 5-HT3 receptor-ion channels; and/or ligands of both proteins may interact with each other’s binding site. In fact Hargreaves et al48 have demonstrated that members of all three major classes of L-type Ca2+ antagonists can reverse the ability of the 5-HT, receptor-selective agonist 1-(m-chlorophenyl)-biguanide to increase intracellular Ca2+ concentration in cell lines that possess either one or both of these Ca2+-ion channels. The latter interaction seems not to be competitive since the binding site for the different classes of L-type Ca2+ channel antagonists appear not to be the same as the serotonin 5-HT, binding site itself (i.e. the orthosteric site) but instead, is an allosteric site in the 5-HT, receptor channel complex. Furthermore, 5-HT release from Enterochromaffin (EC) cells can be prevented by antagonists of both 5-HT3 receptors and LTCCs.48,49 Moreover, human duodenal EC cell exposure to FPL 64176 not only increases intracellular Ca2+ concentration but can also release 5-HT from these cells,50 which is a Ca2+-dependent process.51 These findings provide possible mechanisms via which blockers of both LTCCs and 5-HT3 receptors can mutually pre-

---

**Table 1:** Respective antiemetic ID50 values for amlovidpine and nifedipine against vomiting caused by diverse emetogens.

<table>
<thead>
<tr>
<th>Emetogens</th>
<th>Amlovidpine ID50(mg/kg)</th>
<th>Nifedipine ID50(mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPL 64176</td>
<td>1.10(0.43-2.80)</td>
<td>0.31(0.15-0.62)</td>
</tr>
<tr>
<td>5-HT</td>
<td>2.00(0.80-5.20)</td>
<td>0.22(0.03-1.50)</td>
</tr>
<tr>
<td>2-Me-5-HT</td>
<td>0.65(0.30-1.40)</td>
<td>0.053(0.02-0.17)</td>
</tr>
<tr>
<td>Apomorphine</td>
<td>0.90(0.30-2.60)</td>
<td>0.91(0.32-2.60)</td>
</tr>
<tr>
<td>Quinpirole</td>
<td>2.00(0.78-5.30)</td>
<td>0.10(0.03-0.36)</td>
</tr>
<tr>
<td>Pilocarpine</td>
<td>2.10(0.69-6.20)</td>
<td>nd</td>
</tr>
<tr>
<td>McN-A-343</td>
<td>2.30(0.81-8.50)</td>
<td>0.38(0.06-2.30)</td>
</tr>
<tr>
<td>GR73632</td>
<td>1.37(0.62-3.00)</td>
<td>0.19(0.08-0.43)</td>
</tr>
</tbody>
</table>

*Obtained from Darmani et al 2014 and Zhong et al., 2014.*

Table not determined.
vent the biochemical and behavioral effects of their corresponding selective agonists, including the vomiting behavior.

Indeed, we have further demonstrated that when non-effective antiemetic doses of nifedipine and palonosetron are combined,\(^28\) the combination significantly and in additive manner attenuate both the frequency and the percentage of shrews vomiting in response to either FPL 64176 or 2-Me-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\(_R\) antagonist palonosetron (0.05 or 0.5 mg/kg).\(^33\) 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\(_R\) (and/or possibly NK\(_\text{K}_1\)) with L-type Ca\(^{2+}\) channel-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\(_R\) and NK\(_{\text{K}_1}\)-receptor antagonists both in the laboratory\(^47,52\) and in the clinic.\(^53\) Although in our investigation, the mechanism underlying the additive antiemetic efficacy of combined low doses of L-type Ca\(^{2+}\) channel-antagonists with 5-HT\(_R\) antagonists was not directly studied, the published literature points to their interaction at the signal transduction level involving Ca\(^{2+}\).\(^6,54,55\)

**INTRACELLULAR Ca\(^{2+}\) CHANNELS ANDEMESIS**

The Sarcoplasmic/Endoplasmic Reticulum Ca\(^{2+}\)-ATPase (SERCA) pump is a major mechanism that transports free cytoplasmic Ca\(^{2+}\) into the lumen of Sarcoplasmic/Endoplasmic Reticulum (SER) to fill its internal Ca\(^{2+}\) stores (Figure 1).\(^56-58\) Intracellular Ca\(^{2+}\) release from the SER into the cytoplasm is accomplished by Inositol Trisphosphate Receptors (IP\(_R\)s) and Ryanodine Receptors (RyRs), and this loss is counter-balanced by continuous Ca\(^{2+}\) uptake from the cytoplasm into these SER stores by SERCAs (Figure 1).\(^57\)

**Ca\(^{2+}\)-Mediated Thapsigargin-Evoked Emetic Responses**

The Ca\(^{2+}\) mobilizing agent thapsigargin is a specific and potent inhibitor of SERCA pumps and also causes internal release of stored Ca\(^{2+}\) and consequently a depletion of luminal SER Ca\(^{2+}\) leading to a rise in the free concentration of cytosolic Ca\(^{2+}\) (Figure 1).\(^59-61\) Pharmacological emptying of SER Ca\(^{2+}\) pools by thapsigargin-like drugs can trigger extracellular Ca\(^{2+}\) influx via activation of Store-Operated Ca\(^{2+}\) Entry (SOCE) mediated by Ca\(^{2+}\) Release-Activated Channels (CRAC) and canonical Transient Receptor Potential Channels (TRPC) in non-excitable cells, in which Stromal interacting molecule 1 (STIM1) protein functions as a sensor for Ca\(^{2+}\) store depletion.\(^62-64\) SOCE is also functional in neurons.\(^65\)

Our more recent studies have demonstrated that intraperitoneal administration of thapsigargin (0.1-10 mg/kg, i.p.) can evoke vomiting in the least shrew in a dose-dependent, but bell-shaped manner, with maximal efficacy at 0.5 mg/kg. Such bell-shaped emetic dose-response effect is not unique to thapsigargin since other emetogens may induce a similar dose-response effect.\(^28,66,67\) An important consideration for the emetic effects of thapsigargin is that it augments the cytosolic levels of free Ca\(^{2+}\) in diverse tissues (e.g. muscle, neurons, mast cells, macrophages, etc.). A major role for the involvement of SOCE in the induced emesis can be discounted since the potent and selective SOCE inhibitor YM-58483, only caused a significant

---

*Figure 1: Summary of extracellular and intracellular calcium (Ca\(^{2+}\)) ion-channels and corresponding modulators involved in vomiting. Extracellular Ca\(^{2+}\) influx can increase cytoplasmic Ca\(^{2+}\) levels through numerous Ca\(^{2+}\) channels located in the plasma membrane including emetic L-type Ca\(^{2+}\) channels (LTCCs), serotonin 5-HT\(_R\) receptors (5-HT\(_R\)s) and possibly store-operated Ca\(^{2+}\) channels (SOCE). Cytosolic Ca\(^{2+}\) concentration can be also increased via extracellular luminal release from the sarcoplasmic/endoplasmic reticulum (SER) Ca\(^{2+}\) stores by the SER Ca\(^{2+}\)-ATPase (SERCA) pump (SERCA). Thapsigargin is a specific inhibitor of SERCA and thus enhances cytosolic levels of Ca\(^{2+}\). Examples of blockers/inhibitors of corresponding Ca\(^{2+}\) channels located on the cell membrane (nifedipine, palonosetron and YM-58483, respectively) are also shown.*
reduction in the frequency of thapsigargin-evoked vomiting without providing complete emesis protection (p > 0.05) even at a large dose (10 mg/kg). On the other hand, the LTCC antagonist nifedipine, completely protected 50% of shrews from thapsigargin-evoked vomiting and reduced the mean vomit frequency by 85% at 2.5 mg/kg, whereas its 5 mg/kg dose nearly completely suppressed the vomit frequency and fully protected over 90% of tested shrews. In addition, significant reductions (70-85%) in the frequency of thapsigargin-induced vomiting (but without full emesis protection) were also observed when shrews were pretreated with antagonists of either IP₃Rs (2-APB at 1-2.5, but not 5 mg/kg) or RyRs (dantrolene at 2.5-5 mg/kg)-ER luminal Ca²⁺ 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 2.5 mg/kg dose of nifedipine, led to a complete elimination of thapsigargin-evoked vomiting. The role of the discussed antagonists against the corresponding Ca²⁺ channels and emesis are summarized in Figure 2. Thus, our latest behavioral findings provide in vivo evidence that the SERCA inhibiting agent thapsigargin may enhance cytoplasmic Ca²⁺ concentration via inhibition of cytoplasmic Ca²⁺ uptake in the SER and Ca²⁺ store release through IP₃Rs and RyRs, as well as extracellular Ca²⁺ entry mainly through LTCCs.

Involvement of Ca²⁺ Release Channels in 5-HT₃R-Mediated Emesis

A functional and physical linkage between LTCC and RyRs appear to exist which plays an important role in intracellular Ca²⁺ release following voltage-dependent Ca²⁺ entry through L-type Ca²⁺ channels. We initially determined whether 2-Me-5-HT-induced vomiting can be differentially modulated via manipulation of IP₃Rs and RyRs. We found that the 5-HT₃R-mediated vomiting was insensitive to the IP₃R antagonist 2-APB, but in contrast, was dose-dependently suppressed by the RyR antagonist, dantrolene. Furthermore, a combination of the semi-effective doses of amlodipine and dantrolene, was more potent than each antagonist being tested alone. These behavioral findings suggest that 5-HT₃R stimulation drives extracellular Ca²⁺ through L-type Ca²⁺ channels and 5-HT₃Rs, which leads to Calcium-Induced Calcium-Release (CICR) from intracellular SER stores via RyRs, which greatly amplifies free Ca²⁺ levels in the cytoplasm (Figure 3). These in vivo findings are consistent with previously published in vitro cellular studies demonstrating that 5-HT₃R activation evokes extracellular Ca²⁺ entry which then triggers such Ca²⁺ release from intracellular stores in a RyRs-sensitive manner. The role of the discussed antagonists against the corresponding Ca²⁺ channels and emesis are summarized in Figure 2. Thus, our latest behavioral findings provide in vivo evidence that the SERCA inhibiting agent thapsigargin may enhance cytoplasmic Ca²⁺ concentration via inhibition of cytoplasmic Ca²⁺ uptake in the SER and Ca²⁺ store release through IP₃Rs and RyRs, as well as extracellular Ca²⁺ entry mainly through LTCCs.

CA²⁺-RELATED SIGNALING PATHWAY IN EMESIS

cAMP-PKA

The adenylyl cyclase/cAMP/Protein Kinase A (PKA) signaling pathway can phosphorylate both Ca²⁺ ion channels on plasma membrane and intracellular endoplasmic IP₃ receptors, and respectively increases extracellular Ca²⁺ influx and intracellular Ca²⁺ release. The emetic role of cAMP in the PKA pathway is well established since microinjection of cAMP analogs...
(e.g. 8-bromocAMP) or forskolin (to increase endogenous levels of cAMP) in the area of postrema not only increase electrical activity of local neurons but can also induce vomiting in dogs. Moreover, administration of 8-chlorocAMP in cancer patients produces nausea and vomiting. Furthermore, use of phosphodiesterase inhibitors (such as rolipram) increase cAMP tissue levels, which consequently causes excessive nausea and vomiting in both vomit competent animals and humans. We have also demonstrated that increased 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.

**Ca^2+ /Calmodulin-Dependent Protein Kinase II (CaMKII) and Extracellular Signal-Regulated Protein Kinase (ERK1/2)**

We have established the post-receptor emetic signaling pathway for selective 5-HT_R agonist 2-Me-5-HT in the least shrew. As shown in Figure 3, we have proposed that following 5-HT_R activation, the enhanced Ca^{2+} mobilization is also sequentially linked to the intracellular activation of the CaMKII-ERK1/2 pathway in the brainstem, which plays an important role in 2-Me-5-HT-induced vomiting. In addition, pharmacological elevation of intracellular Ca^{2+} by systemic thapsigargin administration (0.5 mg/kg, i.p.) can also activate the emeticCaMKII-ERK1/2 signaling in the shrew brainstem. Further support for the involvement of CaMKII-ERK1/2 pathway in thapsigargin-evoked vomiting comes from the ability of their specific inhibitors (KN93 and PD98059, respectively) to suppress the induced vomiting in a manner similar to the discussed pathway for the 2-Me-5-HT-induced vomiting. In addition, the low dose combination of nifedipine, 2-APB and dantrolene, which completely abolished thapsigargin-evoked vomiting, also fully suppressed CaMKII-ERK1/2 signaling to basal levels, indicating that elevation in the cytosolic Ca^{2+} concentration is one of the earliest and requisite events in the signal transduction pathways explored in this study (Figure 2). Hence the Ca^{2+}-CaMKII-ERK1/2 emetic cascade in brainstem emetic nuclei may have a common role in the regulation of emetic responses elicited by diverse emetogens. This raises the possibility of novel therapeutic approaches in the prevention of emetic events through strategies targeting specific mechanisms linking Ca^{2+} to downstream intracellular signal transduction system(s).

**CONCLUSION**

In this review, we have discussed: 1) the transmission of emetic signals at the brainstem level is crucially dependent on Ca^{2+} channels located on plasma membrane and intracellular Ca^{2+} stores in the SER; 2) the implications of these findings for the design of novel therapeutic strategies and have compared the role of L-type Ca^{2+} channels antagonists nifedipine with amiodipine in emesis management; and 3) the Ca^{2+}-mediated signaling transduction pathway in the brainstem involved in diverse emetogens-evoked vomiting. We envisage development of universal antiemetics can be possible if one targets: i) one
critical step in each of the few available post-receptor emetic signal transduction systems which the above-discussed diverse emetogens share downstream of their corresponding receptors, or ii) a common essential signal which can cross-talk between these transduction pathways such as, Ca\(^{2+}\).

**CONFLICTS OF INTERESTS**

The authors declare that they have no conflicts of interest.

**REFERENCES**


10. Aman TK, Shen RY, Haj-Dahmane S. D2-like dopamine receptors depolarize dorsal raphe serotonin neurons through the activation of nonselective cationic conductance. *J Pharmacol Exp Therap.* 2007; 320: 376-385. doi: 10.1124/jpet.106.111690


12. Oliveira L, Correia-de-Sa P. Protein kinase A and cak1 (L-type) channels are common targets to facilitatory adenosine and muscarinic m1 receptors on rat motoneurons. *Neurosignals.* 2005; 14: 262-272. doi: 10.1159/000088642

13. Sculptoreano A, Yoshimura N, de Goroat WC, Somogyi GT. Protein kinase C is involved in M1-muscarinic receptor-mediated facilitation of L-type Ca\(^{2+}\) channels in neurons of the major pelvic ganglion of the adult male rat. *Neurochem Res.* 2001; 26: 933-942. doi: 10.1002/A121332500946


20. Rodriguez-Lagunas MJ, Martin-Venegas R, Moreno JJ, Ferrer R. PGE2 promotes Ca\(^{2+}\)-mediated epithelial barrier disrup-


44. Malhotra S, Kumari S, Pandhi P. Effect of calcium antagonists on stress-induced rise in blood pressure and heart rate: a


68. Katoh H, Schlotthauer K, Bers DM. Transmission of information from cardiac dihydropyridine receptor to ryanodine receptor: evidence from BayK 8644 effects on resting Ca(2+) sparks. *Circ Res.* 2000; 87: 106-111. doi: 10.1161/01.RES.87.2.106


