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Review

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

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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 (e.g. 5-HT or 2-Me-5-HT)-, NK_1(GR73632)-, D_2 (apomorphine or quinpirole)-, and M_1 (McN-A343)-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: Gastrointestinaltract; 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_3Rs: Inositol Trisphosphate Receptors; RyRs: Ryanodine Receptors; TRPC: Transient Receptor Potential Channels; SOCE: Store-Operated Ca^{2+} Entry; CRAC: Ca^{2+} Release-Activated Channels; 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.[1] 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).[1] 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.[2] 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.[3] The NK_1 receptor is G-protein coupled and can increase cytoplasmic Ca^{2+} concentration *via* extracellular influx.[3-5] In addition, the 5-HT_3 receptor is a Ca^{2+}-permeable ligand-gated ion channel.[6] 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.[6-9] Other emetogens such as agonists of dopamine D_2,[10,11] cholinergic M_1,[12,13] histaminergic H_1[14,15] and opiate u[16,17]-receptors, as well as cisplatin,[18] prostaglandins,[19,20] 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 (LTCCs) are activated by membrane depolarization, and serve as the principal route of Ca^{2+} entry in electrically excitable cells such as neurons and muscle.[26,27] Our study[28] 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_3R 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_2receptor 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_1-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.

Emetogens	Amlodipine ID_{50}(mg/kg)		Nifedipine ID_{50}(mg/kg)	
	Frequency	Percent inhibition	Frequency	Percent inhibition
FPL 64176	1.10(0.43-2.80)	2.70(1.40- 5.30)	0.31(0.15-0.62)	0.42(0.19-0.90)
5-HT	2.00(0.80-5.20)	3.20(1.60-6.50)	0.22(0.03-1.50)	0.91(0.42-1.90)
2-Me-5-HT	0.65(0.30-1.40)	3.10(1.40-6.60)	0.053(0.02-0.17)	1.34(0.64-2.80)
Apomorphine	0.90(0.30-2.60)	2 .00(0.94-4.30)	0.91(0.32-2.60)	2.02(0.90-4.40)
Quinpirole	2.00(0.78-5.30)	4.40(1.90-10.0)	0.10(0.03-0.36)	0.18(0.09-0.38)
Pilocarpine	2.10(0.69-6.20)	4.60(2.20-9.40)	nd	nd
McN-A-343	2.30(0.61-8.50)	3.20(1.50-7.10)	0.38(0.06-2.30)	0.95(0.43-2.10)
GR73632	1.37(0.62-3.00)	7.10(3.40-14.6)	0.19(0.08-0.43)	0.60(0.28-1.30)

*Obtained from Darmani et al 2014 and Zhong et al., 2014a.[28,33]
nd=not determined.

Table 1: Respective antiemetic ID_{50} values for amlodipine and *nifedipine against vomiting caused by diverse emetogens.

Thus, nifedipine appears to be 2-24 times more potent than amlodipine against vomiting caused by FPL 64176, 5-HT, 2-Me-5-HT, GR73632, quinpirole and McN-A343. 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 amlodipine may not have had sufficient time to reach its sites of action, thus having lower potency. In addition, the positively charged amlodipine associates more slowly with the L-type Ca^{2+} 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 amlodipine in reducing the frequency of cisplatin-induced vomiting by 80%[28,33] could be explained in terms of amlodipine 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 amlodipine against cisplatin-induced vomiting is its ability to bind an additional Ca^{2+} site.[31]

The discussed broad-spectrum antiemetic efficacy of nifedipine and amlodipine 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 patient[40] 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.[43-45] Thus, the broad-spectrum antiemetic potential of both nifedipine and amlodipine against the diverse selective and non-selective emetogens in the least shrew further supports our proposed Ca^{2+} hypothesis and warrants initiation of clinical trials for determination of clinically-useful LTCC antagonist antiemetics.

CROSS-TALK BETWEEN LTCCS AND $5HT_3RS$

Recently we have found that the second generation 5-HT_3 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-HT_3 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 Ca^{2+} through both L-type- and 5-HT_3 receptor-ion channels; and/or ligands of both proteins may interact with each other's binding site. In fact Hargreaves et al[6] have demonstrated that members of all three major classes of L-type Ca^{2+} antagonists can reverse 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 Ca^{2+}-ion channels. The latter interaction seems not to be competitive since the binding site for the different classes of L-type Ca^{2+} channel antagonists appear not to be the same as the serotonin 5-HT_3 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 (EC) cells can be prevented by antagonists of both 5-HT_3 receptors and LTCCs.[48,49] Moreover, human duodenal EC cell exposure to FPL 64176 not only increases intracellular Ca^{2+} concentration but can also release 5-HT from these cells,[50] which is a Ca^{2+}-dependent process.[51] These findings provide possible mechanisms *via* which blockers of both LTCCs and 5-HT_3 receptors can mutually pre-

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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_3R 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_3- (and/or possibly NK_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_3- and NK_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_3R 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_3Rs) 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

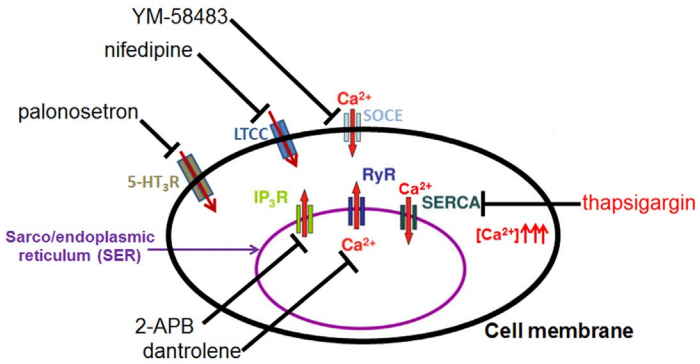

Figure 1: Summary of extracellular and intracellular calcium (Ca^{2+}) ion-channels and corresponding modulators involved in vomiting. Extracellular Ca^{2+}influx can increase cytoplasmic Ca^{2+} levels through numerous Ca^{2+} channels located in the plasma membrane including emetic L-type Ca^{2+} channels (LTCCs), serotonin 5-HT_3 receptors (5-HT_3Rs) and possibly store-operated Ca^{2+} channels (SOCE). Cytosolic Ca^{2+} concentration can be also increased via intracellular luminal release from the sarco/endoplasmic reticulum (SER) Ca^{2+} stores through the inositol triphosphate receptors (IP_3Rs) and ryanodine receptors (RyRs). This luminal Ca^{2+} loss is countere-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+}. Examples of blockers/inhibitors of corresponding Ca^{2+} channels located on the cell membrane (nifedipine, palonosetron and YM-58483, respectively) and on the SER membrane (2-APB and dantrolene, 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 IP_3Rs (2-APB at 1-2.5, but not 5 mg/kg)- or RyRs (dantrolene at 2.5-5 mg/kg)-ER luminal Ca^{2+} release channels. Moreover, while a mixture of 2-APB (1 mg/kg) and dantroline (2.5 mk/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 Ca^{2+} 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 Ca^{2+} concentration *via* inhibition of cytoplasmic Ca^{2+} uptake in the SER and Ca^{2+} store release through IP_3Rs and RyRs, as well as extracellular Ca^{2+} entry mainly through LTCCs.

Involvement of Ca^{2+} Release Channels in 5-Ht_3r-Mediated Emesis

A functional and physical linkage between LTCC and RyRs appear to exist which plays an important role in intracellular Ca^{2+} release following voltage-dependent Ca^{2+} entry through L-type Ca^{2+} channels.[68,69] We initially determined whether 2-Me-5-HT-induced vomiting can be differentially modulated *via* manipulation of IP_3Rs and RyRs.[70] We found that the 5-HT_3R-mediated vomiting was insensitive to the IP_3R 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-HT_3R stimulation drives extracellular Ca^{2+} through L-type Ca^{2+} channels and 5-HT_3Rs, which leads to Calcium-Induced Calcium-Release (CICR) from intracellular SER stores *via* RyRs, which greatly amplifies free Ca^{2+} levels in the cytoplasm (Figure 3). These *in vivo* findings are consistent with previously published *in vitro* cellular studies demonstrating that 5-HT_3R activation evokes extracellular Ca^{2+} entry which then triggers such Ca^{2+} release from intracellular stores in a RyRs-sensitive manner.[8]

CA^{2+}-RELATED SIGNALING PATHWAY IN EMESIS

cAMP-PKA

The adenylyl cyclase/cAMP/Protein Kinase A (PKA) signaling pathway can phosphorylate both Ca^{2+} ion channels on plasma membrane and intracellular endoplasmic IP_3 receptors, and respectively increases extracellular Ca^{2+} influx and intracellular Ca^{2+} release.[71] The emetic role of cAMP in the PKA pathway is well established since microinjection of cAMP analogs

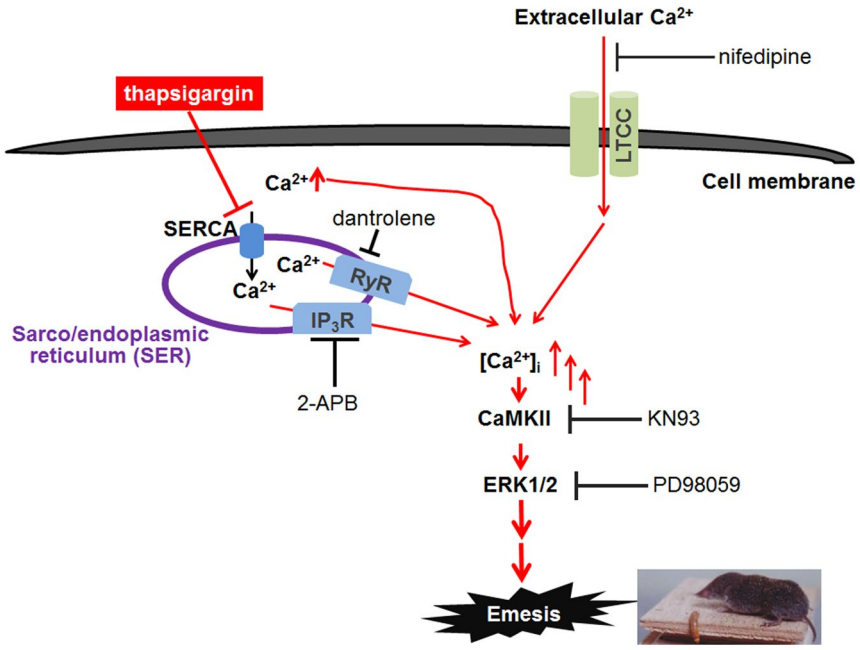

Figure 2: Schemtic model of the proposed Ca^{2+}-CaMKII-ERK1/2 signaling mechanisms in the brainstem underlying thapsigargin-induced emesis in the least shrew. Thapsigargin augments cytoplasmic concentration of Ca^{2+} *via* the: i) inhibition of cytosolic Ca^{2+} uptake into the Endoplasmic Reticulum (ER) by blocking SERCA, ii) release of stored Ca^{2+} from the ER through IP_3Rs and RyRs, and iii) activation of extracellular Ca^{2+} entry mainly through LTCCs. The induced rise in cytosolic Ca^{2+} results in CaMKII activation and subsequent ERK1/2 signaling. The LTCC blocker nifedipine, the RyR antagonist dantrolene, the IP_3R blocker 2-APB, the CaMKII inhibitor KN93, and the ERK inhibitor PD98059, respectively exhibit anti-emetic efficacy against thapsigargin-induced vomiting.

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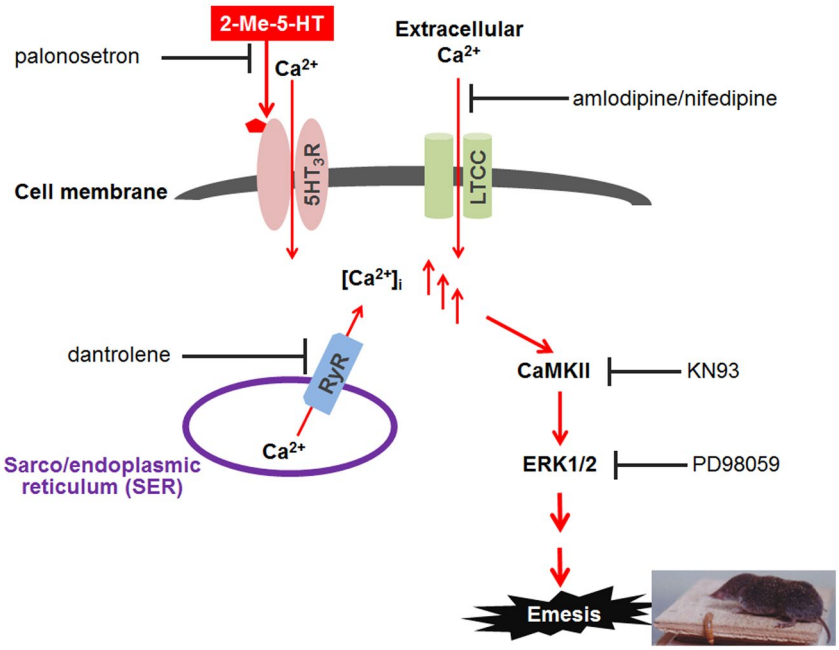

Figure 3: Schemtic model for the proposed Ca^{2+}-CaMKII-ERK1/2 signaling cascade in brainstem underlying 2-Me-5-HT-induced emesis in the least shrew. 5-HT_3R stimulation by the selective agonist 2-Me-5-HT causes an influx of extracellular Ca^{2+} through 5-HT_3Rs/L-type Ca^{2+} channels (LTCCs) which increases the free cytoplasmic concentration of Ca^{2+}, thereby-promoting luminalCa^{2+} release from the endoplasmic reticulum (ER) stores into the cytosol through ryanodine receptors (RyRs) via calcium-induced calcium-release (CICR). This elevation in cellular Ca^{2+} level leads to CaMKII activation and subsequent ERK1/2 signaling. The 5-HT_3R antagonist palonosetron, LTCC blockers amlodipine or nifedipine, the RyR inhibitor dantrolene, the CaMKII inhibitor KN93, and the ERK inhibitor PD98059, respectively exhibit anti-emetic efficacy against 2-Me-5-HT-induced vomiting.

(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.[72] Moreover, administration of 8-chloroc AMP in cancer patients produces nausea and vomiting.[73] 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.[74] 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.[52,54,75]

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_3R agonist 2-Me-5-HT in the least shrew. As shown in Figure 3, we have proposed that following 5-HT_3R 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.[70] 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[76] (Figure 2). 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.[70] 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 amlodipine 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

1. Darmani NA, Ray AP. Evidence for a re-evaluation of the neurochemical and anatomical bases of chemotherapy-induced vomiting. *Chem Rev*. 2009; 109: 3158-3199. doi: 10.1021/cr900117p

2. Seaton G, Hogg EL, Jo J, Whitcomb DJ, Cho K. Sensing change: the emerging role of calcium sensors in neuronal disease. *Semin Cell Dev Biol.* 2011; 22: 530-535. doi: 10.1016/j.semcdb.2011.07.014

3. Suzuki Y, Inoue T, Ra C. L-type Ca^{2+} channels: a new player in the regulation of Ca^{2+} signaling, cell activation and cell survival in immune cells. *Mol Immunol*. 2010; 47: 640-648. doi: 10.1016/j.molimm.2009.10.013

4. Lin YR, Kao PC, Chan MH. Involvement of Ca^{2+} signaling in tachykinin-mediated contractile responses in swine trachea. *J Biomed Sciences.* 2005; 12: 547-558. doi: 10.1007/s11373-005-6796-0

5. Miyano K, Morioka N, Sugimoto T, Shiraishi S, Uezono Y, Nakata Y. Activation of the neurokinin-1 receptor in the rat spinal astrocytes induced Ca^{2+} release from IP3-sensitive Ca^{2+} stores and extracellular Ca^{2+} influx through TRPC3. *Neurochem Int.* 2010; 57: 923-934. doi: 10.1016/j.neuint.2010.09.012

6. Hargreaves AC, Gunthorpe MJ, Taylor CW, Lumis SC. Direct inhibition of 5-hydroxytryptamine3 receptors by antagonists of L-type Ca^{2+} channels. *Mol Pharmacol.* 1996; 50: 1284-1294.

7. Homma K, Kitamura Y, Ogawa H, Oka K. Serotonin induces the increase in intracellular Ca^{2+} that enhances neurite out growth in PC12 cells *via* activation of 5-HT_3 receptors and voltage gated channels. *J Neurosc Res.* 2006; 84: 316-325. doi: 10.1002/jnr.20894

8. Ronde P, Nichols RA. 5-HT_3 receptors induce rises in cytosolic and nuclear calcium in NG108-15 *via* calcium-induced calcium release. *Cell Calcium.* 1997; 22: 357-365. doi: 10.1016/S0143-4160(97)90020-8

9. Takenouchi T, Munekata E. Serotonin increases Ca^{2+} concentration in PC12h cells: effect of tachikinin peptides. *Neurosc Lett.* 1998; 24: 141-144. doi: 10.1016/S0304-3940(98)00253-5

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

11. Wu J, Dougherty JJ, Nichols RA. Dopamine receptor regulation of Ca^{2+} levels in individual isolated nerve terminals from rat striatum: comparison of presynaptic D1-like and D2-like receptors. *J Neuroscience*. 2006; 98: 481-494.

12. Oliveira L, Correia-de-Sa P. Protein kinase A and cav1 (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. Proteinkinase 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: 10.1023/A:1012332500946

14. Barajas M, Andrade A, Hernandez-Hernandez O, Felix R, Arias-Montano J-A. Histamine-induced Ca^{2+} entry in human astrocytoma U373 MG cells: evidence for involvement of store-operated channels. *J Neurosci Res.* 2008; 86: 3456-3468. doi: 10.1002/jnr.21784

15. Yoshimoto K, Hattori Y, Houzen H, Kanno M, Yasuda K. Histamine H_1-receptor-mediated increase in the Ca^{2+} transient without a change in the Ca^{2+} current in electrically stimulated guinea-pig atrial myocytes. *Br J Pharmacol.* 1998; 124: 1744-1750. doi: 10.1038/sj.bjp.0702008

16. Ono T, Inoue M, Rashid MH, Sumikawa K, Ueda H. Stimulation of peripheral nociceptor endings by low dose morphine and its signaling mechanism. *Neurochem Internat.* 2002; 41: 399-407. doi: 10.1016/S0197-0186(02)00047-5

17. Smart D, Hirst RA, Hirota HK, Grandy DK, Lambert DG. The effects of recombinant rat u-opioid receptor activation in CHO cells on phospholipase C, [Ca2+]I and adenylyl cyclase. *Br J Pharmacol*. 1997; 120: 1165-1171. doi: 10.1038/sj.bjp.0701012

18. Splettstoesser F, Florea A-M, Busselberg D. IP3 receptor antagonist, 2-APB, attenuates cisplatin induced Ca^{2+}-influx in Hela-S3 cells and prevents activation of calpain and induction of apoptosis. *Br J Pharmacol.* 2007; 151: 1176-1186. doi: 10.1038/sj.bjp.0707335

19. Almirza WHM, Peters PHJ, van Zoelen EJJ, Theuvenet APR. Role of TRPC channels, Stim1 and Orai1 in PGF2a-induced calcium signaling in NRK fibroblasts. *Calcium Cell.* 2012; 51: 12-21. doi: 10.1016/j.ceca.2011.10.001

20. Rodríguez-Lagunas MJ, Martín-Venegas R, Moreno JJ, Ferrer R. PGE2 promotes Ca^{2+}- mediated epithelial barrier disrup-

tion through EP1 and EP4 receptors in Caco-2 cell monolayers. *Am J Cell Physiol.* 2010; 299: C324-C334.

21. Hagbom M, Sharma S, Lundgren O, Svensson L. Towards a human rotavirus disease model. *Curr Opin Virol.* 2012; 2: 408-418. doi: 10.1016/j.coviro.2012.05.006

22. Hyser JM, Collinson-Pautz MR, Utama B, Estes MK. Rotavirus disrupts calcium homeostasis by NSP4 viroporin activity. 2010; e00265. Available at: http://mbio.asm.org/

23. Poppoff MR, Poulain B. Bacterial toxins and the nervous system: neurotoxins and multipotential toxins interacting with neuronal cells. *Toxins.* 2010; 2: 683-737. doi: 10.3390/toxins2040683

24. Timar Peregrin T, Svensson M, Ahlman H, Jodal M, Lundgren O. The effects on net fluid transport of noxious stimulation of jejunal mucosa in anesthetized rats. *Acta Physiol Scand.* 1999; 166: 55-64.

25. Zuccotti A, Clementi S, Reinbothe T, Torrente A, Vandael DH, Pirone A. Structural and functional differences between l-type calcium channels: crucial issues for future selective targeting. *TIPS.* 2011; 32: 366-375. doi: 10.1016/j.tips.2011.02.012

26. Suzuki Y, Yoshimaru T, Inoue T, Ra C. Ca v 1.2 L-type Ca^{2+} channel protects mast cells against activation-induced cell death by preventing mitochondrial integrity disruption. *Mol Immunol.* 2009; 46: 2370-2380. doi: 10.1016/j.molimm.2009.03.017

27. Yoshimaru T, Suzuki Y, Inoue T, Ra C. L-type Ca^{2+} channels in mast cells: activation by membrane depolarization and distinct roles in regulating mediator release from store-operated Ca^{2+} channels. *Mol Immunol.* 2009; 46: 1267-1277. doi: 10.1016/j.molimm.2008.11.011

28. Darmani NA, Zhong W, Chebolu S, Vaezi M, Alkam T. Broad-spectrum antiemetic potential of the L-type calcium channel antagonist nifedipine and evidence for its additive antiemetic interaction with the 5-HT(3) receptor antagonist palonosetron in the least shrew (Cryptotis parva). *Eur J Pharmacol.* 2014; 722: 2-12. doi: 10.1016/j.ejphar.2013.08.052

29. Zheng W, Rampe D, Triggle DJ. Pharmacological, radioligand binding, and electrophysiological characteristics of FPL 64176, a novel nondihydrpyridine Ca^{2+} channel activator, in cardiac and vascular preparations. *Mol Pharmacol.* 1991; 40: 734-741.

30. Burges RA. The pharmacological profile of amlodipine in relation to ischaemic heart disease. *Postgrad Med J.* 1991; 67(Suppl 3): S9-S15.

31. Burges R, Moisey D. Unique pharmacologic properties of amlodipine. *Am J Cardiol.* 1994; 73: 2A-9A.

32. Toal CB, Meredith PA, Elliott HL. Long-acting dihydropyridine calcium-channel blockers and sympathetic nervous system activity in hypertension: a literature review comparing amlodipine and nifedipine GITS. *Blood Press.* 2012; 21(Suppl 1): S3-S10. doi: 10.3109/08037051.2012.690615

33. Zhong W, Chebolu S, Darmani NA. Broad-spectrum antiemetic efficacy of the L-type calcium channel blocker amlodipine in the least shrew (Cryptotis parva). *Pharmacol Biochem Behav.* 2014a; 120: 124-132. doi: 10.1016/j.pbb.2014.03.005

34. Croom KF, Wellington K. Modified-release nifedipine: a review of the use of modified-release formulations in the treatment of hypertension and angina pectoris. *Drugs.* 2006; 66: 497-528.

35. Meredith PA, Reid JL. Differences between calcium antagonists: duration of action and trough to peak ratio. *J Hypertens.* 1993; 11(Suppl 1): S21-S26.

36. Burges RA, Dodd MG. Amlodipine. *Cardiovasc Drug Rev.* 1990; 8: 25-44.

37. Nayler WG, Gu XH. The unique binding properties of amlodipine: a long-acting calcium antagonist. *J Hum Hypertens.* 1991; 5(Suppl 1): S55-S59.

38. Qu Y-L, Sugiyama K, Hattori K, Yamamoto A, Watanabe K, Nagatoma T. Slow association pf positively charged Ca^{2+} channel antagonist amlodipine to dihhydropyridine receptor sites in the rat brain membranes. *Gen Pharmacol.* 1996; 27: 137-140. doi: 10.1016/0306-3623(95)00085-2

39. Mutoh M, Imanishi H, Torii Y, Tamura M, Saito H, Matsuki N. Cisplatin-induced emesis in Suncus murinus. *Jpn J Pharmacol.* 1992; 58: 321-324.

40. Van Driessche A, Sermigin E, Paemeleire K, van Coster R, Vogelaers D. Cyclic vomiting syndrome: case report and short review of the literature. *Acta Clin Belg.* 2012; 67: 123-126. doi: 10.1016/j.ejpn.2004.11.002

41. Kothare SV. Efficacy of flunarizine in the prophylaxis of cyclical vomiting syndrome and abdominal migraine. *Eur J Paediatr Neurol.* 2005; 9: 23-26. doi: 10.1016/j.ejpn.2004.11.002

42. SamardzicR, Bajcetic M, Beleslin DB. Opposite effects of ethanol and nitrendipine on nicotine-induced emesis and convulsions. *Alcohol.* 1999; 18: 215-219. doi: 10.1016/S0741-8329(99)00005-1

43. Nayler WG. The effect of amlodipine on hypertension-induced cardiac hypertrophy and reperfusion-induced calcium overload. *J Cardiovas Pharmacol.* 1988; 12: S42-S44.

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

double-blind, placebo-controlled study. *Int J Clin Ther*. 2001; 39: 19-24.

45. Mehsen J, Jeppesen P, Erlandsen M, Poulsen PL, Bek T. Lack of effect of short-term treatment with amlodipine and Lisinopril on retinal autoregulation in normotensive patients with type 1 diabetes and mild diabetic retinopathy. *Acta Opthalmol*. 2011; 89: 764-768. doi: 10.1111/j.1755-3768.2009.01847.x

46. Rojas C1, Slusher BS. Pharmacological mechanisms of 5-HT_3 and tachykinin NK_1 receptor antagonism to prevent chemotherapy-induced nausea and vomiting. *Eur J Pharmacol.* 2012; 684(1-3): 1-7. doi: 10.1016/j.ejphar.2012.01.046

47. Darmani NA, Chebolu S, Amos B, Alkam T. Synergistic antiemetic interactions between serotonergic 5-HT_3- and tachykininergic NK_1-receptor antagonists in the least shrew (Cryptotis parva). *Pharmacol Biochem Behav.* 2011; 99: 573-579. doi: 10.1016/j.pbb.2011.05.025

48. Minami M, Endo T, Hirafugi M, et al. Pharmacological aspects of anticancer drug-induced emesis with emphasis on serotonin release and vagal nerve activity. *Pharmacol Therapeut.* 2003a; 99: 149-165. doi: 10.1016/S0163-7258(03)00057-3

49. Minami M, Taquchi S, Kikuchi T, et al. Effects of fluvoxamine, a selective serotonin re-uptake inhibitor, on serotonin release from the mouse isolated ileum. *Res Commun Mol Pathol Pharmacol.* 2003b; 113-114: 115-131.

50. Lomax RB, Gallego S, Novalbos J, Garcia AG, Warhurst G. L-type calcium channels in enterchromaffin cells from guinea pig and human duodenal crypts: an in situ study. *Gastroenterology.* 1999; 117: 1363-1369. doi: 10.1016/S0016-5085(99)70286-6

51. Racke K, Reimann A, Schworer H, Kilbinger H. Regulation of 5-HT release from enterochromaffin cells. *Behav Brain Res.* 1996; 73: 83-87.

52. Darmani NA, Zhong W, Chebolu S, Mercadante F. Differential and additive suppressive effects of 5-HT_3 (palonosetron)- and NK_1 (netupitant)-receptor antagonists on cisplatin-induced vomiting and ERK1/2, PKA and PKC activation. *Pharmacol Biochem Behav.* 2015; 131: 104-111. doi: 10.1016/j.pbb.2015.02.010

53. Warr D. Management of highly emetogenic chemotherapy. *Curr Opin Oncol.* 2012; 24: 371-375. doi: 10.1097/CCO.0b013e328352f6fb

54. Darmani NA, Dey D, Chebolu S, Amos B, Kandpal R, Alkam T. Cisplatin causes over-expression of tachykinin NK(1) receptors and increases ERK1/2- and PKA-phosphorylation during peak immediate- and delayed-phase emesis in the least shrew (Cryptotis parva) brainstem. *Eur J Pharmacol*. 2013; 698: 161-169. doi: 10.1016/j.ejphar.2012.09.008

55. Stathis M, Pietra C, Rojas C, Slusher BS. Inhibition of substance P-mediated responses in NG108-15 cells by netupitant and palonosetron exhibit synergistic effects. *Eur J Pharmacol.* 2012; 689: 25-30. doi: 10.1016/j.ejphar.2012.05.037

56. Garaschuk O, Yaari Y, Konnerth A. Release and sequestration of calcium by ryanodine-sensitive stores in rat hippocampal neurons. *J Phys.* 1997; 502: 13-30.

57. Gómez-Viquez L, Guerrero-Serna G, García U, Guerrero-Hernández A. SERCA pump optimizes Ca^{2+} release by a mechanism independent of store filling in smooth muscle cells. *Biophys J.* 2003; 85: 370-380. doi: 10.1016/S0006-3495(03)74481-6

58. Gómez-Viquez NL, Guerrero-Serna G, Arvizu F, García U, Guerrero-Hernández A. Inhibition of SERCA pumps induces desynchronized RyR activation in overloaded internal Ca^{2+} stores in smooth muscle cells. *Am J Physiol Cell Physiol.* 2010; 298: C1038-C1046. doi: 10.1152/ajpcell.00222.2009

59. Beltran-Parrazal L, Fernandez-Ruiz J, Toledo R, Manzo J, Morgado-Valle C. Inhibition of endoplasmic reticulum Ca^{2+} ATPase in preBötzinger complex of neonatal rat does not affect respiratory rhythm generation. *Neuroscience.* 2012; 224: 116-124. doi: 10.1016/j.neuroscience.2012.08.016

60. Michelangeli F, East JM. A diversity of SERCA Ca^{2+} pump inhibitors. *Biochem Soc Trans.* 2011; 39: 789-797. doi: 10.1042/BST0390789

61. Solovyova N, Verkhratsky A. Neuronal endoplasmic reticulum acts as a single functional Ca^{2+} store shared by ryanodine and inositol-1,4,5-trisphosphate receptors as revealed by intra-ER [Ca2+] recordings in single rat sensory neurones. *Pflugers Arch*. 2003; 446: 447-454.

62. Cheng KT, Ong HL, Liu X, Ambudkar IS. Contribution and regulation of TRPC channels in store-operated Ca^{2+} entry. *Curr Top Membr.* 2013; 71: 149-179. doi: 10.1016/B978-0-12-407870-3.00007-X

63. Feske S. Calcium signaling in lymphocyte activation and disease. *Nat Rev Immunol.* 2007; 7: 690-702. doi: 10.1038/nri2152

64. Parekh AB, Putney JW Jr. Store-operated calcium channels. *Physiol Rev.* 2005; 85: 757-810. doi: 10.1152/physrev.00057.2003

65. Moccia F, Zuccolo E, Soda T, et al. Stim and Orai proteins in neuronal Ca(2+) signaling and excitability. *Front Cell Neurosci.* 2015; 9: 153.

66. Bhandari P, Bingham S, Andrews PL. The neuropharmacology of loperamide-induced emesis in the ferret: the role of the area postrema, vagus, opiate and 5-HT_3 receptors. *Neuropharmacology.* 1992; 31: 735-742. doi: 10.1016/0028-3908(92)90034-M

67. Wynn RL, Essien E, Thut PD. The effects of different anti-emetic agents on morphine-induced emesis in ferrets. *Eur J Pharmacol.* 1993; 241: 47-54. doi: 10.1016/0014-2999(93)90931-7

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

69. Resende RR, da CJL, Kihara AH, Adhikari A, Lorencon E. Intracellular Ca^{2+} regulation during neuronal differentiation of murine embryonal carcinoma and mesenchymal stem cells. *Stem Cells Dev.* 2010; 19: 379-394. doi: 10.1089/scd.2008.0289

70. Zhong W, Hutchinson TE, Chebolu S, Darmani NA. Serotonin 5-HT_3 Receptor-Mediated Vomiting Occurs *via* the Activation of Ca^{2+}/CaMKII-Dependent ERK1/2 Signaling in the Least Shrew (Cryptotis parva). *PLoS One*. 2014b; 9: e104718. doi: 10.1371/journal.pone.0104718

71. Yao L, Fan P, Jiang Z, Gordon A, Mochly-Rosen D, Diamond I. Dopamine and ethanol cause translocation of ePKC associated with eRACK: Cross-talk between cAMP-dependent protein kinase A and protein kinase c signaling pathways. *J pharmacol Exp Therap.* 2008; 73: 1105-1112. doi: 10.1124/mol.107.042580

72. Carpenter DO, Briggs DB, Knox AP, Strominger N. Excitation of area postrema neurons by transmitters, peptides and cyclic nucleotides. *J Neurophysiol.* 1988; 59: 358-369.

73. Propper DJ, Saunders MP, Salisbury AJ, et al. Regulation of 5-HT release from enterochromaffin cells. *Behav Brain Res*. 1996; 73: 83-87.

74. Mori F, Perez-Torres S, De Caro R, et al. The human area postrema and other nuclei related to the emetic reflex express cAMP phosphor diesterases4B and 4D. *J Chem Neuroanatomy.* 2010; 40: 36-42.

75. Alkam T, Chebolu S, Darmani NA. Cyclophosphamide causes activation of protein kinase A (PKA) in the brainstem of vomiting least shrews (Cryptotis parva). *Eur J Pharmacol.* 2014; 722: 156-164. doi: 10.1016/j.ejphar.2013.09.080

76. Zhong W, Chebolu S, Darmani NA. Thapsigargin-induced activation of Ca^{2+}-CaMKII-ERK in brainstem contributes to substance P release and induction of emesis in the least shrew. *Neuropharmacology* (in press). 2016.