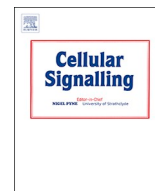




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Review

GPCR mediated control of calcium dynamics: A systems perspective

Vaibhav Dhyani^a, Suman Gare^a, Rishikesh Kumar Gupta^a, Sarpras Swain^a, K.V. Venkatesh^b, Lopamudra Giri^{a,*}

^a Department of Chemical Engineering, Indian Institute of Technology Hyderabad, Sangareddy, Telangana, India

^b Department of Chemical Engineering, Indian Institute of Technology Bombay, Powai, Mumbai, Maharashtra, India



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ABSTRACT

G-protein coupled receptor (GPCR) mediated calcium (Ca^{2+})-signaling transduction remains crucial in designing drugs for various complex diseases including neurodegeneration, chronic heart failure as well as respiratory diseases. Although there are several reviews detailing various aspects of Ca^{2+} -signaling such as the role of IP_3 receptors and Ca^{2+} -induced- Ca^{2+} -release, none of them provide an integrated view of the mathematical descriptions of GPCR signal transduction and investigations on dose-response curves. This article is the first study in reviewing the network structures underlying GPCR signal transduction that control downstream $[\text{Ca}_c^{2+}]$ -oscillations. The central theme of this paper is to present the biochemical pathways, as well as molecular mechanisms underlying the GPCR-mediated Ca^{2+} -dynamics in order to facilitate a better understanding of how agonist concentration is encoded in Ca^{2+} -signals for G_{α_q} , G_{α_s} , and $\text{G}_{\alpha_{i/o}}$ signaling pathways. Moreover, we present the GPCR targeting drugs that are relevant for treating cardiac, respiratory, and neuro-diseases. The current paper presents the ODE formulation for various models along with the detailed schematics of signaling networks. To provide a systems perspective, we present the network motifs that can provide readers an insight into the complex and intriguing science of agonist-mediated Ca^{2+} -dynamics. One of the features of this review is to pinpoint the interplay between positive and negative feedback loops that are involved in controlling intracellular $[\text{Ca}_c^{2+}]$ -oscillations. Furthermore, we review several examples of dose-response curves obtained from $[\text{Ca}_c^{2+}]$ -spiking for various GPCR pathways. This paper is expected to be useful for pharmacologists and computational biologists for designing clinical applications of GPCR targeting drugs through modulation of Ca^{2+} -dynamics.

1. Introduction

G-protein-coupled receptors (GPCRs) are the targets of approximately 40% of all pharmaceutical drugs [1]. Activation of GPCRs is known to trigger a cascade of events that lead to the modulation of cytosolic Ca^{2+} -dynamics [2]. Pharmacological compounds targeting GPCRs can be used to modulate of Ca^{2+} -signaling in order to treat pro-inflammatory diseases [3,4]. Specifically, it has been reported that cancer cells can be selectively killed and/or arrested by targeting Ca^{2+} -

channels [5]. The role of GPCR mediated Ca^{2+} -signaling in chronic interstitial lung diseases, such as idiopathic pulmonary fibrosis and scleroderma has been reviewed by Janssen et al. [6]. In the context of the recent COVID-19 pandemic, Zhou et al. [7] suggested that angiotensin receptor blockers may have the potential of inhibiting viral entry and can be considered as a prospective repurposable drug for 2019-nCoV/SARS-CoV-2. Many therapeutics used to either enhance bronchodilation or prevent bronchoconstriction for treating respiratory diseases including asthma are known to target β_2 -adrenergic receptors

Abbreviations: $[\text{Ca}_c^{2+}]$, Cytosolic calcium concentration; $[\text{Ca}_{ER}^{2+}]$, Endoplasmic reticulum calcium concentration; AC, Adenyl cyclase; ATP, Adenosine triphosphate; AUC, Area under the curve; C5a, Complement component 5a; Ca^{2+} , Calcium; cAMP, Cyclic adenosine monophosphate; CHIP, C-terminus of Hsp70-Interacting Protein; CHO, Chinese hamster ovary; CICR, Calcium-induced-calcium-release; CXCR4, C-X-C Motif Chemokine Receptor 4; DAG, Diacylglycerol; ER, Endoplasmic reticulum; ERK, Extracellular signal-regulated kinase; FRET, Fluorescence resonance energy transfer; GPCR, G-protein coupled receptor; GRK, G protein-coupled receptor kinase; HEK, Human embryonic kidney; IP_3 , Inositol trisphosphate; IP_3R , Inositol trisphosphate-receptor; ISI, Inter-spike interval; LCC, L-type calcium-channel; mGluR, Metabotropic glutamate receptor; ODE, Ordinary differential equation; PIP_2 , Phosphatidylinositol 4,5-bisphosphate; PKC, Protein kinase C; PLC, Phospholipase C; PMCA, Plasma membrane calcium-ATPase; RyR, Ryanodine receptor; SERCA, Sarco/endoplasmic reticulum Ca^{2+} -ATPase; SR, Sarcoplasmic reticulum; SRM, Selective reaction monitoring; STIM, Stromal interaction molecule; VGCC, Voltage-gated calcium channel

* Corresponding author at: Department of Chemical Engineering, Indian Institute of Technology-Hyderabad, Kandi, Sangareddy District, Telangana 502285, India.

E-mail address: giril@che.iith.ac.in (L. Giri).

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[8,9] and modulate Ca^{2+} -signaling [10]. In this context, a more profound knowledge of the role and mechanism through which cytosolic Ca^{2+} is regulated by GPCRs can help in curing these diseases.

Ca^{2+} -dysregulation is known to be associated with many diseases in muscular and nervous systems. For example, in end-stage heart failure, the reduced level of sarcoplasmic reticulum Ca^{2+} -ATPase (SERCA) expression leads to disturbed Ca^{2+} -homeostasis [11]. Recent evidence also indicates that neuronal Ca^{2+} -signaling is abnormal in many neurodegenerative disorders such as Alzheimer's, Huntington disease, and Parkinson's disorder [12].

Within a wide variety of cells, Ca^{2+} serves as an almost universal ionic messenger, delivering signals received at the cell surface to the inside of the cell. These signals are controlled by the concentration of the modulating agonists and encoded in the complex spatiotemporal behavior of cytosolic Ca^{2+} -concentration ($[\text{Ca}_c^{2+}]$), ranging from stochastic spiking to regular oscillations, and more complex waveforms [13]. Ca^{2+} -signals regulate the intracellular processes operating over a wide time range, from neurotransmission in microseconds to gene transcription at a scale of minutes to hours [14]. Cells can quickly raise

or reduce the $[\text{Ca}_c^{2+}]$ through tight regulation mediated through GPCRs, Ca^{2+} -stores, channels, pumps, and exchangers. GPCR mediated $[\text{Ca}_c^{2+}]$ -oscillations are known to control a wide range of cellular functions amid and including cell division to apoptosis [15,16]. While in excitable cells, these functions include muscle contraction [17] and neurotransmitter release [18] in non-excitabile cells, Ca^{2+} regulates transcription [19] and cell cycle progression [20]. For pancreatic β -cells, Ca^{2+} -dynamics control the changes in insulin secretion [21]. Vital cellular functions like fertilization, development, differentiation, adhesion, growth, secretion, platelet activation, gene expression, and memory are also linked to Ca^{2+} -signaling regulated by GPCRs [22,23]. Ca^{2+} mediates these functions by $[\text{Ca}_c^{2+}]$ -sensing effectors that translate Ca^{2+} -signals with varying spatiotemporal dynamics into specific cellular responses [24]. In this scenario, the recent scientific efforts are focusing on drug screening and testing based on the quantification of Ca^{2+} -signatures over conventional biochemical assays [13,25].

Several review papers have been published in the last three decades on the function and structure of GPCR targeting drugs as well as bio-molecular details of Ca^{2+} -signaling [26–28]. Many of them focus on

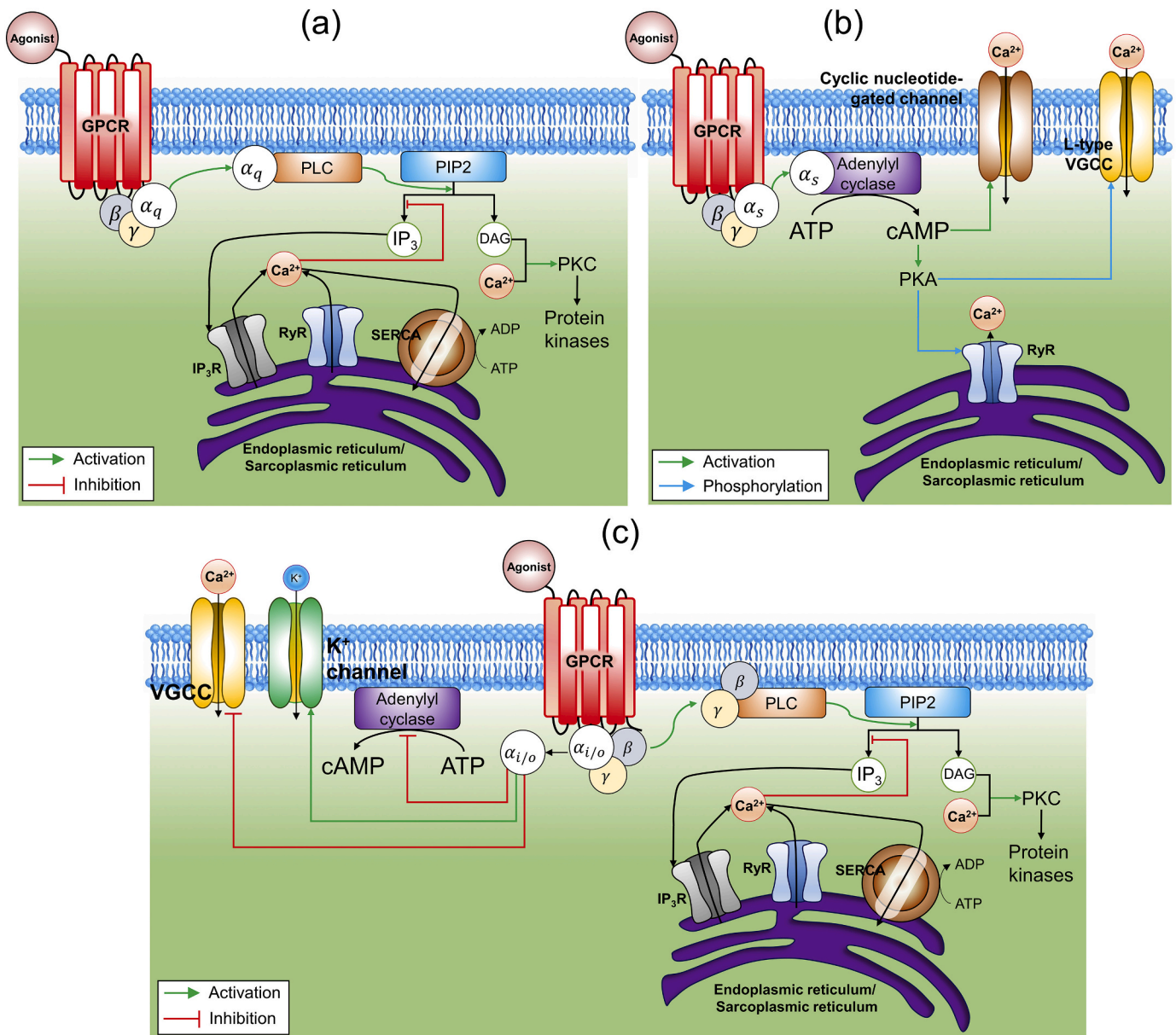


Fig. 1. Biochemical pathway and molecular details of controlling intracellular Ca^{2+} -dynamics through: (a) $G_{\alpha q}$, (b) $G_{\alpha s}$, and (c) $G_{\alpha i/o}$ coupled receptors.

providing a quantitative description of Ca²⁺-toolbox and a thorough understanding of non-linear coupled systems [27–30]. One of these review articles characterizes the biophysical and biochemical mechanisms of Ca²⁺-signaling in astrocytes [27]. However, the review does not elucidate the interplay between positive and negative feedback loops yielding oscillations and how GPCR targeting agonists may encode various levels of frequencies. Another review emphasizes the key features of modeling of Ca²⁺-induced-Ca²⁺-release (CICR) through inositol triphosphate (IP₃)-receptors (IP₃R) and ryanodine receptors (RyR) and how local Ca²⁺-release affects global oscillations [30]. The role of Ca²⁺-entry through store-operated channels in controlling Ca²⁺-signaling is emphasized in Dupont et al. [31]. However, none of these reviews address how GPCR targeting drugs modulate the Ca²⁺-dynamics and their relevance in perspectives of pharmacology. Here, we propose a single bridging article encompassing the details of dose-response curves obtained from [Ca_c²⁺]-spiking for various GPCR pathways along with the systems of ordinary differential equations (ODEs) and network motifs. To the best of our knowledge, this is the first instance of the review that combines experimental investigations as well as theoretical studies on GPCR mediated Ca²⁺-dynamics.

In this review, we provide the current perspectives of GPCR targeting drugs that are relevant for treating cardiac, respiratory, and neuro-diseases along with agonist-mediated encoding of Ca²⁺-dynamics corresponding to G_{αq}, G_{αs}, and G_{αi/o} signaling. Secondly, we emphasize the systems perspectives through the mathematical representation of ODEs that describe GPCR mediated Ca²⁺-dynamics and the underlying feedback loops. Encoding of amplitude and frequency

being the two crucial features of information processing in cells, we provide a detailed analysis of network motifs present in the GPCR mediated circuits. This article also attempts to encompass the details of how the agonist concentration is encoded by Ca²⁺-signals. The detailed schematics of biomolecular pathways and mathematical functions presented may serve as a starting point for the construction of computational models and simulation of the dose-response curve for linking them to experimental data. Finally, this article reviews prominent examples of GPCR targeting agonist concentration-response curves of [Ca_c²⁺]-spiking found from experiments.

2. Role of GPCRs in regulating [Ca_c²⁺]-response

GPCRs are the heptahelical receptors on the plasma membrane that are coupled to guanine nucleotide-binding proteins (G-proteins) within the cell. The binding of the agonist to the extracellular domain of the GPCR activates the receptor and leads to a conformational change on the intracellular side of the membrane. Subsequently, the G-protein (that has three subunits, α, β, and γ) is activated in response to this conformational change. Based on the type of G-protein's α-subunit activated, the GPCRs can be classified into three major types, G_{αq}, G_{αs}, and G_{αi/o} coupled receptors, where each type has its own signal transduction mechanism (Fig. 1). Various examples of GPCRs that are known to regulate Ca²⁺-signaling through G_{αq}, G_{αs}, or G_{αi/o} pathways are presented in Table 1. Representative FDA approved GPCR targeting drugs used to treat some of the respiratory, cardiovascular, and nervous system diseases has also been shown in Fig. 2. Many of these GPCR

Table 1
Examples of G_{αq}, G_{αs}, and G_{αi/o} coupled GPCRs along with various agonists and antagonists involved in the regulation of Ca²⁺-dynamics [33–41].

GPCR type	Subtypes	Mechanism	Agonist		Antagonist	
			Compound	Application	Compound	Application
mGluR	Group I (mGluR _{1/5})	G _{αq}	▶ DHPG	Neuroprotection	▶ YM 298,198	Antinociception
	Group II (mGluR _{2/3})	G _{αi/o}	▶ Biphenylindanone A	Anxiolytic and antipsychotic	▶ LY-341,495	Generalized anxiety disorder
	Group III (mGluR _{5/6/8})		▶ L-AP4	Neuroprotection	▶ MAP4	Blocking L-AP4-induced depression
GABA _B	GABA _A	G _{αi/o}	▶ β-hydroxy-GABA	Anticonvulsant	▶ Bicuculline	Causes convulsions (no medical application)
	GABA _B		▶ Baclofen	Treatment of muscle spasticity	▶ Saclofen	Inhibits baclofen binding
Adrenergic	α ₁	G _{αq}	▶ Noradrenaline	Treatment of low blood pressure	▶ Acetylpromazine	Antipsychotic
			▶ Oxymetazoline, Xylometazoline	Treatment of nasal congestion		
	α ₂	G _{αi/o}	▶ Agmatine	Treating depression and nerve pain	▶ Phentolamine	Control of hypertension induced by pheochromocytomas
	β ₁	G _{αs}	▶ Isoprenaline	Treatment of bradycardia	▶ Metoprolol	Treatment of high blood pressure and chest pain
			▶ Formoterol	Treatment of asthma		
β ₂	▶ Salmeterol	Treatment of COPD	▶ Timolol			
β ₃		▶ Amibegron	Antidepressant and anxiolytic	SR 59230A	Hyperthermia treatment	
Muscarinic acetylcholine receptors	M ₁	Predominantly G _{αq}	▶ Carbachol	Treating glaucoma	▶ Ipratropium bromide	Symptomatic treatment of chronic obstructive pulmonary disease and asthma
	M ₂	G _{αi/o}				
	M ₃	G _{αq}	▶ Oxotremorine	Antipsychotic effect	▶ Aclidinium bromide	COPD
	M ₄	G _{αi/o}				
	M ₅	G _{αq}				
Opioid	δ ₁ , δ ₂ , κ ₁ , κ ₂ , κ ₃ , μ ₁ , μ ₂ , μ ₃ , ORL ₁ , ζ	G _{αi/o}	Etorphine, levorphanol, meperidine, methadone, etc.	Analgesic	Diprenorphine, naloxone, naltrexone, β-CAN, etc.	Reverse the effects of opioid analgesics
Somatostatin	SST ₁ , SST ₂ , SST ₃ , SST ₄ , SST ₅	G _{αi/o}	Octreotide acetate	Treating acromegaly	⁶⁴ Cu-NODAGA-JR11	Targeted radionuclide therapy
Vasopressin	V _{1A} OR (V ₁)	G _{αq}	▶ Vasopressin	Ventricular tachycardia and ventricular defibrillation	▶ Conivaptan	Treatment of heart failure
	V _{1B} OR (V ₃)					
	V ₂	G _{αs}			▶ Tolvaptan	

▶ : For neuro/nerve related disorders, ▶ : for heart diseases, ▶ : for lung/respiratory diseases
 Yellow : G_{αq} pathway, Purple : G_{αi/o} pathway, Green : G_{αs} pathway,

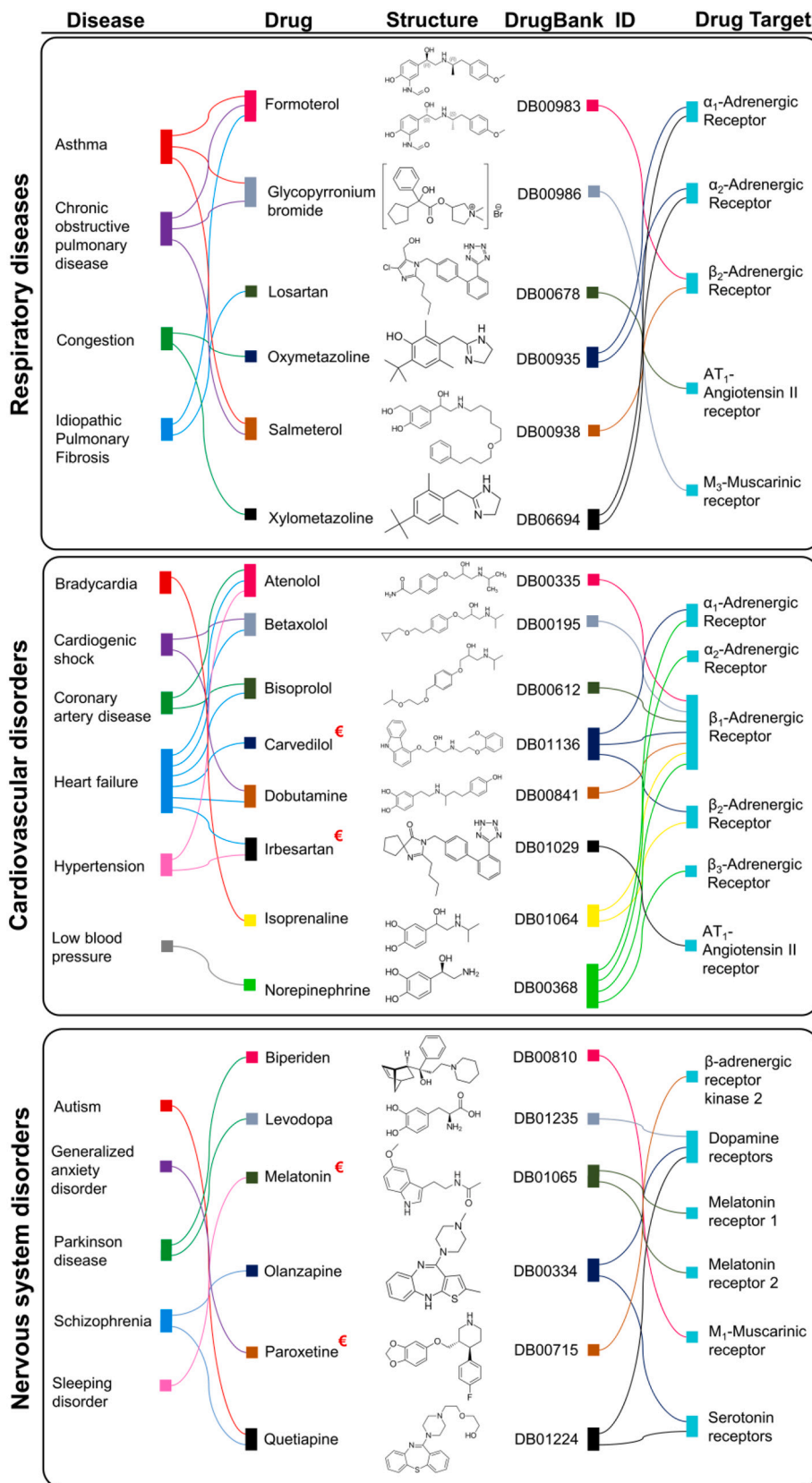


Fig. 2. FDA approved GPCR targeting drugs corresponding to respiratory, cardiovascular, and nervous system diseases. This diagram highlights the relation between various diseases and GPCR targeting drugs. €: GPCR targeting drugs suggested by Zhou et al. [7] for possible treatment of 2019-nCoV/SARS-CoV-2.

targeting drugs such as irbesartan are known to modulate the $[Ca_c^{2+}]$ -levels and thereby assumes significance in the treatment of cardiac complications [32].

In the G_{α_q} pathway, when the ligand binds to the GPCR, the α_q

subunit of G-protein is activated which further activates phospholipase C (PLC) which is an enzyme restricted to the plasma membrane. Once activated, PLC catalyzes the conversion of a phosphatidylinositol 4,5-bisphosphate (PIP₂), a phospholipid component of the cell membrane,

into IP₃ and diacylglycerol (DAG). IP₃ being soluble in the cytoplasm diffuses through the cell and binds to IP₃-receptor (IP₃R) on the surface of the endoplasmic reticulum/sarcoplasmic reticulum (ER/SR), leading to the transportation of Ca²⁺ from ER/SR into the cytoplasm. Additionally, Ca²⁺ and DAG together work to activate protein kinase C (PKC), which further phosphorylates other molecules, leading to altered cellular activity [29,42]. As per several mechanistic models, [Ca_c²⁺] provides positive feedback on IP₃ either directly [43,44] or indirectly via stimulation of PLC [45] (Fig. 1(a)). Paroxetine (G_{αq}) is known to induce apoptosis through an increase in intracellular Ca²⁺ and the generation of reactive oxygen species (ROS) [46].

In the G_{αs} pathway, α_s subunit of G-protein activates adenylyl cyclase (AC), which leads to the production of cyclic adenosine monophosphate (cAMP). cAMP further leads to the activation of protein kinase A (PKA) (Fig. 1(b)). PKA is known to phosphorylate several proteins that regulate excitation-contraction coupling through L-type Ca²⁺-channel (LCC), ryanodine receptor (RyRs), and myosin binding protein C. It has been shown that the activation of β-adrenergic stimulation may enhance SR Ca²⁺-release through phosphorylation of LCCs, or due to phosphorylation of LCCs and RyRs [47,48].

In the G_{αi/o} pathway, α_{i/o} subunit of G-protein is known to inhibit the cAMP production as well as voltage-gated Ca²⁺-channels (VGCCs). In contrast to the G_{αq} pathway, the PLC pathway is activated here by βγ subunit (Fig. 1(c)). Melatonin (G_{αi/o}) is known to fine-tune intracellular Ca²⁺ and eliminate myocardial damage through IP₃R/mitochondrial uniporter pathway [49].

Although there have been extensive studies on G_{αq} mediated [Ca_c²⁺]-oscillation [50,51], there are few investigations for G_{αi/o} mediated [Ca_c²⁺]-oscillation. Recent studies on drug testing framework reveals G_{αi/o} (α₂-adrenergic receptor and CXCR4) mediated modulation of Ca²⁺-signaling in HeLa cells [52]. Also, there are investigations where G_{αs} mediated signaling enhances local Ca²⁺-concentrations in cardiomyocytes [47]. Although it is not known exactly if there is a specific pattern present for the [Ca_c²⁺]-oscillations corresponding to G_{αi/o}, G_{αq}, and G_{αs} pathways, here we show examples of Ca²⁺-responses from each group. Fig. 3(a)-(b) show vasopressin and acetylcholine (G_{αq}) mediated [Ca_c²⁺]-oscillations. The pattern obtained by activation of the α₂-adrenergic receptor (G_{αi/o}) has been shown in Fig. 3(c)-(e). Fig. 3(f) shows how activation of the β-adrenergic receptor (G_{αs}) modulates the Ca²⁺-signaling and how it is different from vasopressin mediated regulation. These curves present the agonist concentration encoding in Ca²⁺-signals for G_{αi/o}, G_{αq}, and G_{αs} signaling.

3. Signal transduction: GPCR mediated regulation of [Ca_c²⁺]-oscillations

The mechanisms underlying the complex Ca²⁺-waveforms have lured both theoretical and experimental researchers in the past three decades. In general, deciphering these mechanisms through investigations based on experiments remains challenging due to the heterogeneity present on the oscillation patterns [57]. Thurley et al. [58] have offered a solution by relative frequency encoding instead of encoding its absolute value. It has been shown that despite the variability in the inter-spike-interval (ISI), steps in agonist concentration causes the stochastic period of the ISI to change by the same factor in all the cells. These fold changes reliably encode changes in agonist concentration, and they result in an exponential dependence of average ISI on the concentration of agonist. Hence, [Ca_c²⁺]-spikes enable reliable signaling in a cell population despite randomness and heterogeneity present in a population.

To date, the major modeling approach to investigate the [Ca_c²⁺]-oscillations in cells is based on numerical solutions of the system of differential equations (Table 2) [29]. Here, we describe two of these models to explain how computational modeling can be used to yield agonist-mediated [Ca_c²⁺]-response.

In 2000, Kummer et al. [59] developed a model to show the

dynamic behavior of [Ca_c²⁺] with different agonist stimuli (Fig. 4(a)-(b)). They illustrated the shift from simpler [Ca_c²⁺]-spiking to complex and chaotic time profile with agonist stimulation which was denoted by the parameter k_2 in their ODE system. Unlike other models, they considered receptor-specific self-enhanced behavior of the activated G_α subunits and a slow negative feedback of [Ca_c²⁺] that inhibit the receptor (Fig. 5(a)). Further, the IP₃ concentration was not considered a separate variable but was taken to be dependent on the dynamics of activated PLC.

The mathematical structure of the model can be given as,

$$\frac{d[G_\alpha]}{dt} = k_1 + k_2[G_\alpha] - \frac{k_3[G_\alpha][PLC]}{[G_\alpha] + k_4} - \frac{k_5[G_\alpha][Ca_c^{2+}]}{[G_\alpha] + k_6} \quad (1)$$

$$\frac{d[PLC]}{dt} = k_7[G_\alpha] - \frac{k_8[PLC]}{[PLC] + k_9} \quad (2)$$

$$\begin{aligned} \frac{d[Ca_c^{2+}]}{dt} &= \frac{k_{10}[Ca_c^{2+}][PLC][Ca_{ER}^{2+}]}{[Ca_{ER}^{2+}] + K_{11}} + k_{12}[PLC] + k_{13}[G_\alpha] - \frac{k_{14}[Ca_c^{2+}]}{[Ca_c^{2+}] + k_{15}} - \frac{k_{16}[Ca_c^{2+}]}{[Ca_c^{2+}] + k_{17}} \end{aligned} \quad (3)$$

$$\frac{d[Ca_{ER}^{2+}]}{dt} = \frac{k_{10}[Ca_c^{2+}][PLC][Ca_{ER}^{2+}]}{[Ca_{ER}^{2+}] + k_{11}} + \frac{k_{16}[Ca_c^{2+}]}{[Ca_c^{2+}] + k_{17}} \quad (4)$$

Their model was compared with experimental data obtained from hepatocytes stimulated with phenylephrine (G_{αq}) and adenosine triphosphate (ATP), but no parameter estimation was performed. Later on, in 2004, Larsen et al. [60] modified the model proposed by Kummer et al. [59] to illustrate the encoding and decoding of Ca²⁺-signals based on the cooperativity of Ca²⁺-binding to various proteins. The major objective for performing such modification is to include the role of agonist in encoding frequency, amplitude, and waveform of the [Ca_c²⁺]-oscillations. The network structure of this model is presented in Fig. 5(b). Larsen et al. [60] also introduced the role of the mitochondria in controlling the [Ca_c²⁺]-oscillations (Table 2).

Another model focusing on PKC mediated phosphorylation of GPCR and PLC-β mediated Ca²⁺-transport has been proposed by De Pittà et al. [61] in 2009. Their model describes glutamate-mediated [Ca_c²⁺]-oscillations in astrocytes. The network structure and feedback loops for this model are presented in Fig. 5(d).

In 2014, Giri et al. [54] proposed a model that included the translocation of G-protein subunits and the role of various γ subunits in controlling [Ca_c²⁺]-oscillation. They used the framework developed by Kummer et al. [59] and performed the modifications to include the PLC activation by G_{βγ} (specific to G_{αi/o} pathway) as well as spatial redistribution of active G_{βγ} between the plasma membrane and internal membrane (Fig. 4(c)-(d)). They developed a two-γ subunit models by considering different translocation rates of γ subunits present in HeLa cells. The mathematical structure of the model is presented in Eqs. (5)–(10) and the network motifs are presented in Fig. 5(d).

$$\begin{aligned} \frac{d[\beta\gamma_{fast\ PM}]}{dt} &= K_a m_1 ([R_0] + [R]) - k_1 \frac{[\beta\gamma_{fast\ PM}]}{K m_1 + [\beta\gamma_{fast\ PM}]} (PLC - \beta) \\ &- k_2 \frac{[Ca_c^{2+}][\beta\gamma_{fast\ PM}]}{[K m_2 + [\beta\gamma_{fast\ PM}]]} - k_{if} ([\beta\gamma_{fast\ PM}] - [\beta\gamma_{fast\ IM}]) \end{aligned} \quad (5)$$

$$\begin{aligned} \frac{d[\beta\gamma_{slow\ PM}]}{dt} &= K_a m_1 ([R_0] + [R]) - k_1 \frac{[\beta\gamma_{slow\ PM}]}{K m_1 + [\beta\gamma_{slow\ PM}]} [PLC] \\ &- k_2 \frac{[Ca_c^{2+}][\beta\gamma_{slow\ PM}]}{[K m_2 + [\beta\gamma_{slow\ PM}]]} - k_{is} ([\beta\gamma_{slow\ PM}] - [\beta\gamma_{slow\ IM}]) \end{aligned} \quad (6)$$

$$\frac{d[\beta\gamma_{fast\ IM}]}{dt} = k_{if} ([\beta\gamma_{fast\ PM}] - [\beta\gamma_{fast\ IM}]) \quad (7)$$

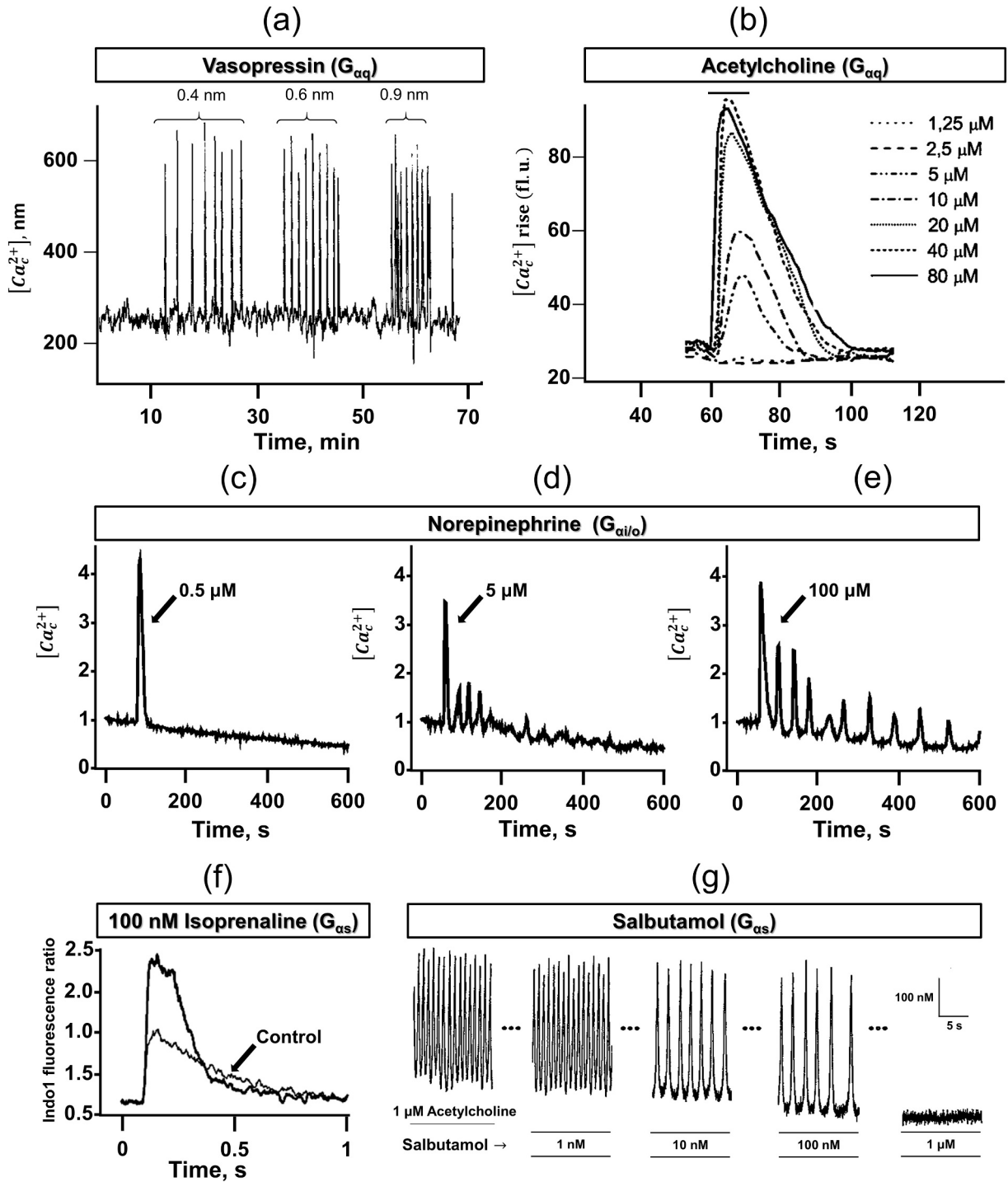


Fig. 3. Agonist concentration encoding of Ca^{2+} -dynamics, (a) $[Ca_c^{2+}]$ -oscillations in a single rat hepatocyte induced by the stimulation of the vasopressin receptor (G_{aq} pathway). An increase in drug dose shows an increase in the frequency of $[Ca_c^{2+}]$ -spikes. (b) Modulation of amplitude and area under the curve (AUC) of $[Ca_c^{2+}]$ -spikes by increasing the drug dose of acetylcholine (G_{aq} pathway). (c)-(e) Frequency and amplitude modulation of $[Ca_c^{2+}]$ -oscillation in a single representative HeLa cell by stimulation of α_2 -adrenergic receptor ($G_{ai/o}$ pathway). Various doses of norepinephrine were used. (f) Increase in Ca^{2+} -release (amplitude modulation) by treatment of β -adrenergic receptor (G_{as} pathway) using isoprenaline in rat atrial and ventricular myocytes. (g) Frequency modulation of $[Ca_c^{2+}]$ -oscillation by treatment of β_2 -adrenergic receptors (G_{as} pathway) with increasing doses of salbutamol in porcine airway smooth muscle cells. Over a concentration range of 1 nM to 100 nM, salbutamol progressively decreased the frequency of acetylcholine-induced $[Ca_c^{2+}]$ -transients but did not alter the amplitude of the spike train. At salbutamol concentration of 1 μ M, the oscillations were completely inhibited. Adapted with permission from [50,53–56]. Copyright (2020).

$$\frac{d[\beta\gamma_{slowIM}]}{dt} = k_{ts}([\beta\gamma_{slowPM}] - [\beta\gamma_{slowIM}])$$

$$(8) \quad \frac{d[PLC - \beta]}{dt} = k_3[\beta\gamma_{PM}] - \frac{k_4[PLC - \beta]}{[PLC - \beta] + k_{m_3}} \quad (10)$$

$$\beta\gamma_{PM} = [\beta\gamma_{fastPM}] + [\beta\gamma_{slowPM}]$$

$$(9) \quad \text{To depict the differential translocation rates, they assumed a fast gamma subunit } \gamma_{fast} \text{ and slow gamma subunit as } \gamma_{slow} \text{ where } k_{fj} \text{ and } k_{ts}$$

Table 2
Description of the major model variables, Ca^{2+} -channel/receptor details, and feedback loops present in the ODE systems corresponding to single-cell models yielding GPCR mediated $[Ca_c^{2+}]$ -oscillations.

Model	Channels considered	Drug	Cell line/ type	ODE variables	Across plasma membrane		Across endoplasmic reticulum		Across mitochondria		Feedback loops
					J_{in}	J_{out}	$J_{IP_3} + J_{leak} + J_{CICR}$	J_{SMCA}	J_{in}	J_{out}	
Kummer et al. (2000) [59]	IP ₃ R, SERCA, Ca ²⁺ -ATPase, Ca ²⁺ entry channel on plasma membrane	ATP	Hepatocyte	[G _a], [PLC], [Ca _c ²⁺], [Ca _{ER} ²⁺]	$k_{12}[PLC] + k_{13}[G_a]$	$-\frac{k_{14}[Ca_c^{2+}]}{[Ca_c^{2+}] + k_{15}}$	$J_{IP_3} + J_{leak} + J_{CICR}$	$-\frac{k_{16}[Ca_c^{2+}]}{[Ca_c^{2+}] + k_{17}}$	—	—	-ve feedback of [Ca _c ²⁺] on IP ₃ R and G _a at high concentrations +ve feedback of [Ca _c ²⁺] on IP ₃ R
Larsen et al. (2004) [60]	IP ₃ R, SERCA, Ca ²⁺ -ATPase, Ca ²⁺ entry channel on plasma membrane, MCU, NCLX	—	—	[G _a], [PLC], [Ca _c ²⁺], [Ca _{ER} ²⁺], [Ca _{mit} ²⁺]	$k_{12}[PLC] + k_{13}[G_a]$	$-\frac{k_{14}[Ca_c^{2+}]}{[Ca_c^{2+}] + k_{15}}$	$\frac{k_{10}[Ca_c^{2+}][PLC]^4}{[PLC]^4 + K_4^4} - [Ca_c^{2+}]$	$-\frac{k_{16}[Ca_c^{2+}]}{k_{17}^8 + [Ca_c^{2+}]}$	$\frac{k_{20}[Ca_c^{2+}]}{[Ca_c^{2+}] + k_{21}} - [Ca_c^{2+}]$	$-\frac{k_{18}[Ca_c^{2+}]^8}{k_{19}^8 + [Ca_c^{2+}]^8}$	-ve feedback of [Ca _c ²⁺] on IP ₃ R and G _a at high concentrations +ve feedback of [Ca _c ²⁺] on IP ₃ R
De Pitià et al. (2009) [61]	IP ₃ R, SERCA, Leak channel on ER	Glutamate	Astrocytes	[Ca _c ²⁺], [Ca _{ER} ²⁺], [Ca _{ER} ²⁺], [IP ₃]	—	—	$r_2 m_{\infty}^3 n_{\infty}^3 h^3 ([Ca_{ER}^{2+}] - [Ca_c^{2+}]) + r_1 ([Ca_{ER}^{2+}] - [Ca_c^{2+}])$	$-\frac{v_{ER}[Ca_c^{2+2}]}{K_{ER}^2 + [Ca_c^{2+}]^2}$	—	—	+ve and -ve feedback of [Ca _c ²⁺] on IP ₃ R
Giri et al. (2014) [54]	IP ₃ R, SERCA, Ca ²⁺ -ATPase	Norepinephrine	HeLa	[G _a], [PLC], [Ca _c ²⁺], [Ca _{ER} ²⁺]	$k_{12}[PLC]$	$-\frac{k_7[Ca_c^{2+}]}{[Ca_c^{2+}] + Km_6}$	$k_6 \frac{[Ca_{ER}^{2+}]}{Km_4 + [Ca_{ER}^{2+}]}$	$-\frac{k_8[Ca_c^{2+}]}{[Ca_c^{2+}] + Km_5}$	—	—	-ve feedback of [IP ₃] on IP ₃ production -ve feedback of [Ca _c ²⁺] on IP ₃ R and G _a at high concentrations +ve feedback of [Ca _c ²⁺] on IP ₃ R

[G_a] is the concentration of G_s subunit, [PLC] is the concentration of PLC. [Ca_c²⁺], [Ca_{ER}²⁺] and [Ca_{mit}²⁺] refer to Ca²⁺ concentration in the cytoplasm, endoplasmic reticulum, and mitochondria respectively.

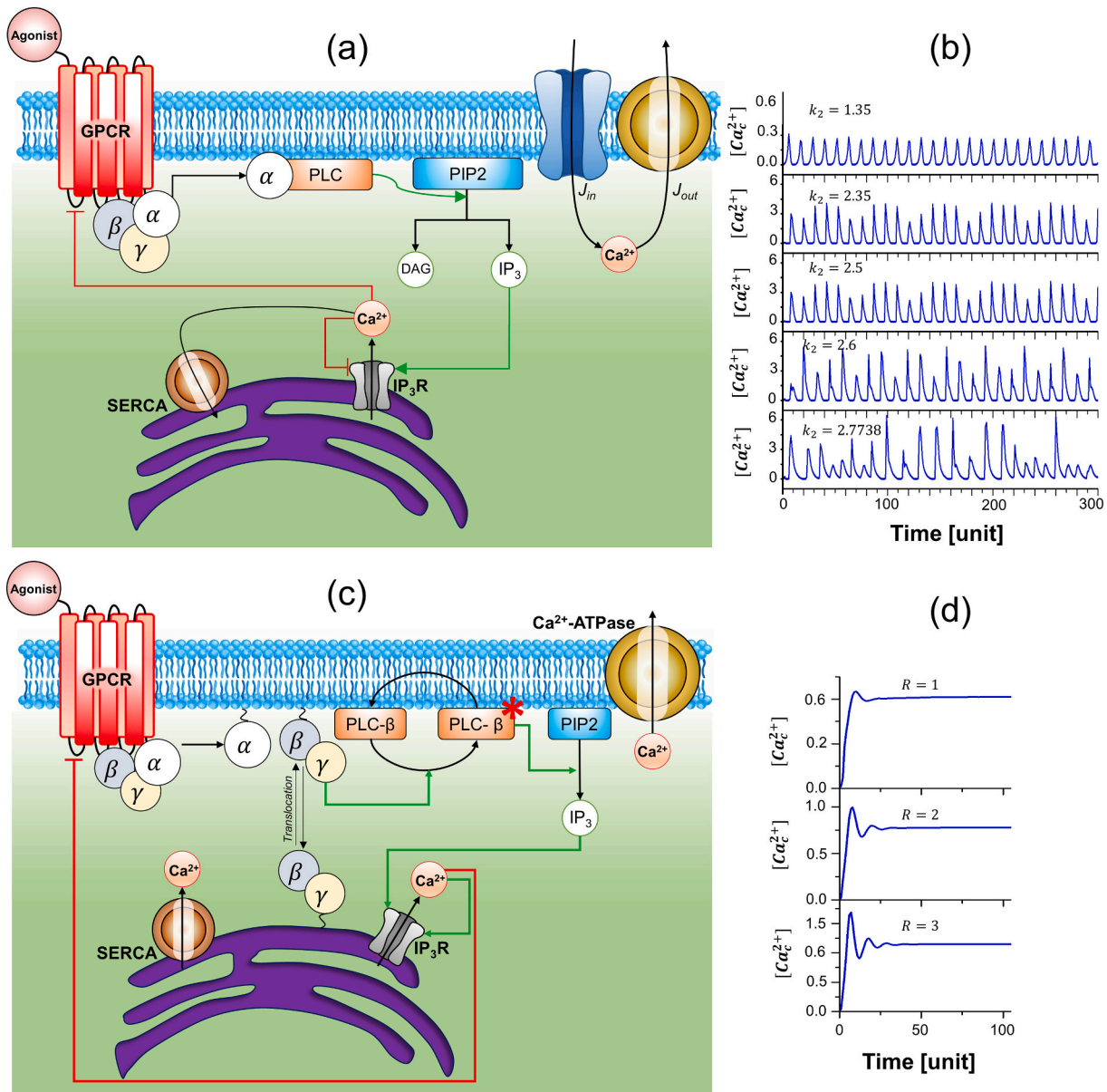


Fig. 4. (a) Schematic representation of GPCR mediated Ca^{2+} -signaling pathway and network structure present in Kummer et al. [59], (b) Switching from simple to chaotic $[\text{Ca}_c^{2+}]$ -oscillations with increasing agonist stimulation have been shown with the change in the value of the parameter k_2 in Kummer et al. [59] (see Eq. (1)), (c) Schematic representation of Ca^{2+} -signaling pathway and network structure present in the model proposed by Giri et al. [54], (d) Changes in the amplitude and frequency of $[\text{Ca}_c^{2+}]$ -oscillations with increasing agonist concentration have been shown with the change in the value of parameter R in Giri et al. [54] (see Eq. (5)).

are the rate constants for translocation of fast and slow γ -subunits. Eq. (5) and (6) represent the active fast and slow gamma subunits at the plasma membrane and Eq. (7) and (8) represent the active fast and slow gamma subunits at the internal membrane. Eq. (10) represents the rate kinetics of $[\text{PLC}\beta]$ which is analogous to Eq. (2) in the model proposed by Kummer et al. [59]. The $[\text{Ca}_c^{2+}]$ and $[\text{Ca}_{ER}^{2+}]$ dynamics in their model follow Eqs. (3) and (4). The parameter R in Eq. (5) and (6) represents the agonist concentration which modulates the $[\text{Ca}_c^{2+}]$ -oscillations (as shown in Fig. 5(d)). Although their model did not include the dynamics of mitochondrial Ca^{2+} -oscillations and the details of the downstream reactions involved in the decoding process, it was validated with experimental results collected by simultaneous imaging of the protein translocation and $[\text{Ca}_c^{2+}]$ -oscillation.

Next, we present a detailed analysis of network motifs for a set of theoretical models describing GPCR mediated control of Ca^{2+} -dynamics (Fig. 5a-f). The schematic representation of primary circuits containing inflow/outflow structure and feedback structures reveal

how the interplay between positive and negative feedback loops gives rise to a well-defined $[\text{Ca}_c^{2+}]$ -oscillation. For example, studies have shown the fast positive feedback and a slow negative feedback can be defined to control the frequency and amplitude of the oscillations resulting in varied responses [54]. Such feedback structures can be useful for identifying the particular combinations of motifs that will exhibit specific types of oscillations with the GPCR targeting agonist as the stimuli. As explained in the previous section, IP_3 - Ca^{2+} cross-coupling is a key feature of the GPCR pathways. The repetitive Ca^{2+} -release and reuptake by intracellular stores are based on positive and negative feedback of $[\text{Ca}_c^{2+}]$ on the IP_3 or IP_3R . Such biphasic feedback of Ca^{2+} on the IP_3R is a minimum requirement to generate Ca^{2+} -transients (Table 2). However, there can be multiple positive feedback loops (Fig. 5) present in the system that are needed for better control of frequency from the perspective of particular physiology. For example, additional positive feedback loops like autocatalytic feedback may give rise to chaotic oscillations (Fig. 5(a)) [59]. Moreover, translocation

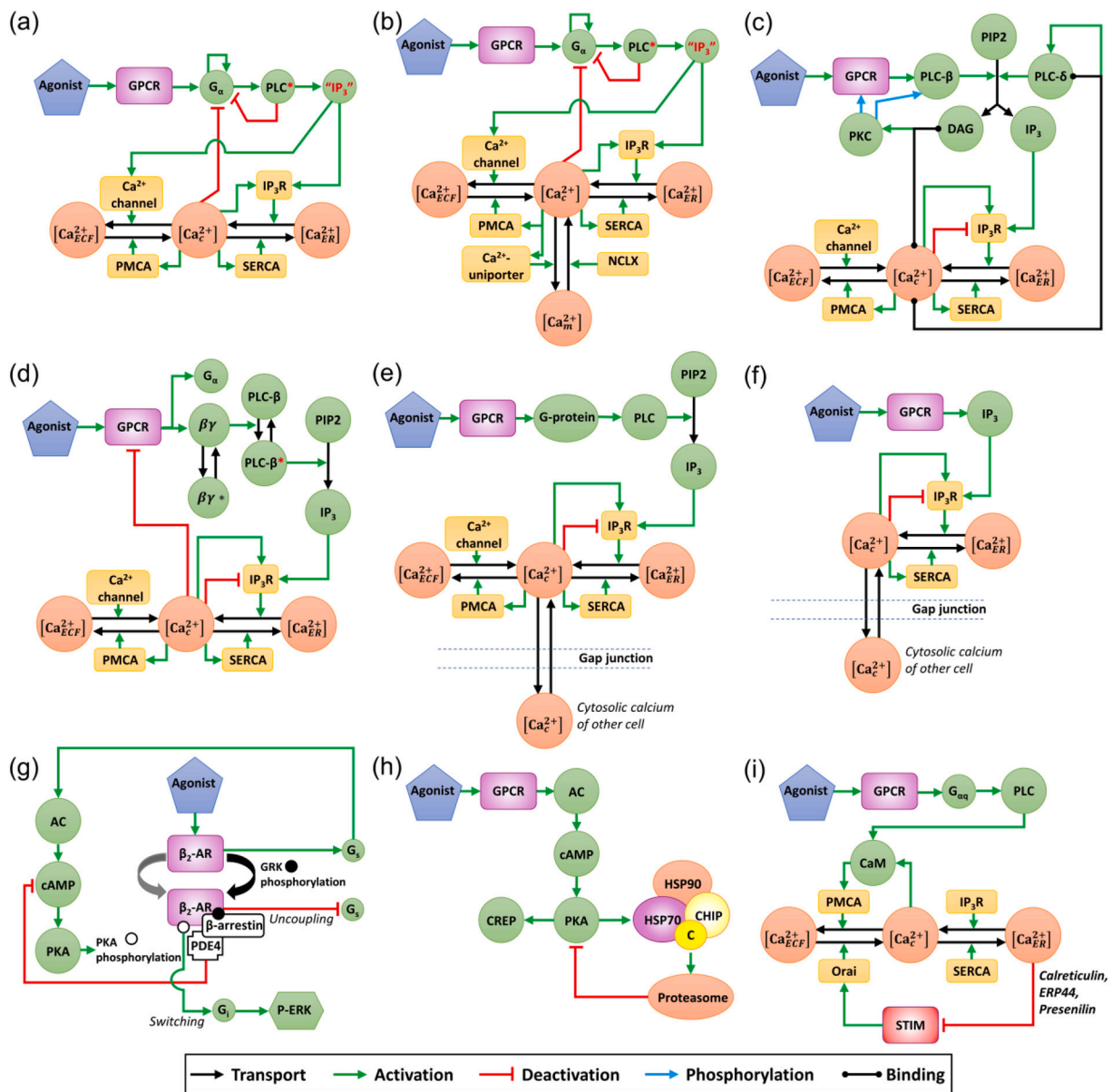


Fig. 5. Schematic representation of primary circuits containing positive and negative feedback loops present in the GPCR signaling cascade systems reported in: (a) Kummer et al. [59] (phenylephrine ($G_{\alpha q}$) and ATP ($G_{\alpha s}$)-mediated Ca^{2+} -response in hepatocyte cell line), (b) Larsen et al. [60], (c) De Pittà et al. [61] (glutamate ($G_{\alpha q}$)-mediated Ca^{2+} -response in astrocytes), (d) Giri et al. [54] (norepinephrine ($G_{\alpha i/o}$)-mediated Ca^{2+} -response in HeLa cell line), (e) Sun et al. [64] (histamine ($G_{\alpha q}$)-mediated Ca^{2+} -response in HUVEC cells), (f) Potter et al. [65] (ATP ($G_{\alpha q}$) mediated Ca^{2+} -response in NIH-3 T3/MDA-MB-231 cells), (g) Baillie et al. [66] (β -Arrestin-mediated switching of β -adrenergic receptors from $G_{\alpha s}$ to $G_{\alpha i/o}$ in HEK cells), (h) Rinaldi et al. [67] (CHIP mediated inhibition of PKA in forskolin ($G_{\alpha s}$)-stimulated mouse embryonic fibroblast), and (i) Abell et al. [68] (Ca^{2+} -influx mediated by STIM ($G_{\alpha q}$) in drosophila cells). Note that various circuits exhibit different configurations of positive and negative feedback loops. The specific characteristics are motifs with inflow and outflow, fast positive feedback, negative feedback, adaptive negative feedback, and diffusible intermediates.

coupled with reactions may lead to damping in oscillations (Fig. 4(d),5(d) Giri et al. [54]).

The detailed investigation of the models depicting receptor-mediated Ca^{2+} -dynamics shows that the circuits include four types of feedback including positive feedback of agonist on PLC [54,59,60], Ca^{2+} -feedback on the IP_3R (Ca^{2+} activation and inhibition of the IP_3R) [61], positive feedback of Ca^{2+} on PLC [62], and negative feedback of Ca^{2+} on IP_3 by activation of IP_3 -kinase [63]. Table 2 shows the distribution of these loops in literature on the modeling of Ca^{2+} -dynamics. Similarly, the schematic representation of network structures shown in Fig. 5 reveals both similarities and dissimilarities between different models.

Although there are some computational investigations indicating

the presence of feedback structures corresponding to GPCR signaling network, limited investigations focus on gathering experimental data proving these structures. Here, we discuss the recent progress in experimental investigations on various feedback inhibition on receptors present in the circuit. An experimental report showed that extracellular signal-regulated kinase (ERK)-mediated regulation of G protein-coupled receptor kinase (GRK) is involved in establishing negative feedback on GPCR (β_2 -AR, G_s) through receptor phosphorylation (Fig. 5(g)) [66]. Moreover, a recent report showed the GPCR mediated cAMP elevation leads to activation of PKA and release of catalytic subunit C (PKAc) [67]. Further, the recruitment of chaperone bound ligase that mediates ubiquitylation and proteolysis of PKAc, acts as a negative feedback for restricting PKA activities (Fig. 5(h)) [67]. Along with modeling studies,

Giri et al. [54] show the experimental evidence of $\beta\gamma$ translocation triggered by G_i -coupled receptor activation, that leads to damping of $[Ca_c^{2+}]$ -oscillation (Fig. 5(d)). Another report shows the presence of parallel adaptive feedback in G_q -coupled signaling using RNAi to knockdown SERCA, plasma membrane Ca^{2+} -ATPase (PMCA), and stromal interaction molecule (STIM) and lowering of expression (Fig. 5(i)) [68]. The protein concentration was measured by selective reaction monitoring mass spectrometry (SRM). The results showed that levels of these proteins are regulated by multiple feedbacks so that change in cytosolic and ER Ca^{2+} slowly adjusts the amount of signaling components. Parallel adaptive feedback may act with other Ca^{2+} -signaling mechanisms such as RyRs to reconfigure local Ca^{2+} -dynamics. A novel Ca^{2+} -sensor elucidates that in case of malignant hyperthermia, RyR mediated Ca^{2+} -leak alters the local Ca^{2+} -dynamics in human skeletal muscle [69]. A combination of whole-cell physiology and Ca^{2+} -imaging shows that dysfunction of neurons during diseased conditions may be due to a deleterious positive feedback loop associated with $[Ca_c^{2+}]$ mediated enhanced mGluR1 (G_q) function [70]. Some of these circuits are shown to elucidate the presence of negative feedback and adaptive negative feedback present in the GPCR signaling network (Fig. 5 (g)-(i)).

4. $[Ca_c^{2+}]$ -signature: a biomarker for drug screening

Recent advancements in imaging techniques such as confocal and two-photon microscopy have led to the measurement of the spatio-temporal oscillations in $[Ca_c^{2+}]$ with high precision and resolution. This makes Ca^{2+} -flux assay as a novel tool in GPCR drug screening [35]. Specifically, Ca^{2+} -imaging offers a platform for high-content data acquisition and optimal drug-dose screening. It has been shown that confocal imaging-based assays can be used for measurement of response at a higher spatial resolution that can be further used for classification

of responses using k -means clustering and ranking of drugs [52]. Since there are a significant number of potential candidate drugs for the treatment of cardiac diseases, neuro-diseases and respiratory complications (Fig. 2), Ca^{2+} -imaging and systems pharmacology based platforms can be used for testing various drugs and drug-drug interactions. Moreover, Ca^{2+} -imaging offers a phenotypic drug discovery paradigm [71]. Additionally, fluorescence resonance energy transfer (FRET) based Ca^{2+} -imaging has been shown as an attractive alternative for the identification of novel Alzheimer's disease therapeutics [72].

During drug screening and selection of doses, generally, $[Ca_c^{2+}]$ -oscillations are measured for a large range of agonist concentrations. Since we aim to present the systems perspective of the GPCR mediated Ca^{2+} -signaling network, we present some examples of dose-response curves of $[Ca_c^{2+}]$ -spiking. Such response curves applying to several GPCR pathways have been investigated in [50,52,58,73]. These dose-response curves are the mathematical representation of GPCR mediated signal transduction. There have been several studies in which dose-response of GPCR targeting agonist is presented in terms of area under the curve (AUC) and amplitude of $[Ca_c^{2+}]$ -response [50,73]. However, there are rather fewer investigations where the dose-response curve is constructed based on agonist-mediated frequency encoding [51]. In this scenario, we present three examples of dose-response curves for specific agonists that are used as neuromodulators (Fig.6). Saxena et al. [51] reported the dose-response curve that uses the number of $[Ca_c^{2+}]$ -spikes obtained through stimulation of serotonin_{1A} receptor ($G_{\alpha q}$ coupled) in CHO cells (Fig. 6(a)). In another study [50], the dose-response curve with respect to the amplitude of acetylcholine ($G_{\alpha q}$) mediated $[Ca_c^{2+}]$ -oscillation has been reported (Fig. 6(b)). An example of dose-response of 2-chloro-N6-cyclopentyladenosine targeting Adenosine-A1 receptor ($G_{\alpha i/o}$ coupled) in terms of AUC has also been shown in Fig. 6(c).

The construction of such a dose-response curve and analysis of

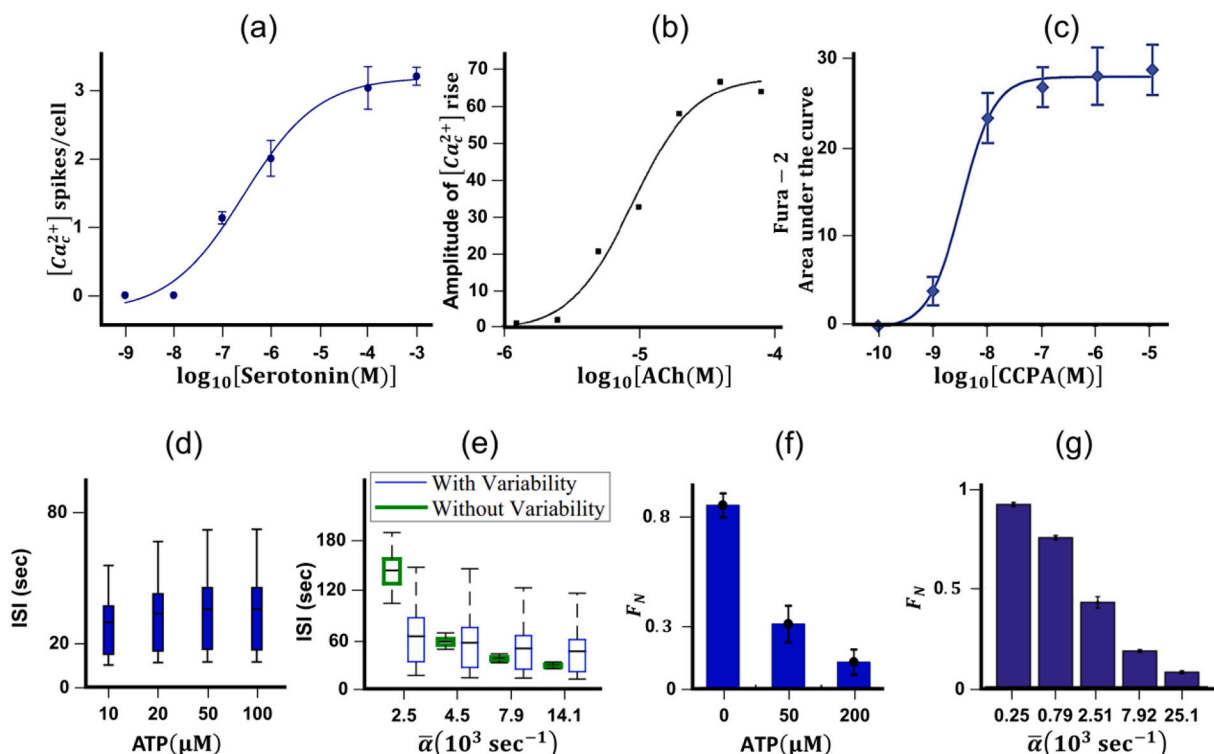


Fig. 6. Dose-response characteristics of $[Ca_c^{2+}]$ -spiking for various GPCR targeting agonists: Experimental results and model predictions (a) Dose-response curve of $[Ca_c^{2+}]$ -spikes via. Stimulation of serotonin_{1A} receptor ($G_{\alpha q}$ coupled) in CHO cells [51]. (b) Dose-response curve with respect to the amplitude of acetylcholine mediated $[Ca_c^{2+}]$ -oscillation in Neuro2a cells ($G_{\alpha q}$ pathway) [50], (c) Dose-response curve with respect to AUC of $[Ca_c^{2+}]$ -oscillation evoked by stimulation of Adenosine-A1 receptor ($G_{\alpha i/o}$ pathway) by 2-chloro-N6-cyclopentyladenosine in HEK293 cells [73]. Comparison of experimental and simulated dose-response with respect to ISI (d)-(e) and the fraction of non-oscillating cells (f)-(g) in ATP mediated $[Ca_c^{2+}]$ -oscillations in NIH-3 T3/MDA-MB-231 cells [65]. Copyright (2020).

$[Ca_c^{2+}]$ -oscillations can provide valuable insights into drug potential, which can further aid in the faster in-vitro screening of drugs [52,74]. Although some of the experimental studies have been conducted to obtain the dose-response curves for GPCR targeting drugs, there are very few investigations on simulation of drug dose-dependent encoding of $[Ca_c^{2+}]$ -oscillation [54,59]. Especially, the predicted dose-response curves for GPCR targeting agonists obtained from simulations are not validated with experimental results. Mourya et al. [75] proposed a model for prediction of the dose-response for complement component 5a (C5a) in Raw 264.7 cells where parameter estimation for the dose-response curve was performed using sensitivity analysis. Simulated results for $[Ca_c^{2+}]$ -amplitude were in agreement with experimental values at various doses of C5a. The dose-response was found to be ultrasensitive since multiple steps are involved in the signaling cascade starting from C5a receptor activation to Ca^{2+} release [75]. In 2014, Long-Fei Li et al. [76] developed another model to study the dose-response for Ca^{2+} -flux in spermatozoa where the proposed model gives the transient Ca^{2+} -responses to various concentrations of progesterone. Parameters were estimated by fitting the experimental data and the simulated dose-response curve was in agreement with the Ca^{2+} -dynamics observed in experiments. However, while attempting a match between Ca^{2+} -dynamics in a cell population, cell-to-cell variability remains the main reason for discrepancies between experiment and model results. In order to address this issue, a distribution in drug concentration was assumed for obtaining a qualitative match between simulation and experimental data on the dose-response of norepinephrine in HeLa cells [54]. In this study α_2 -adrenergic receptor was activated at three different doses of norepinephrine (Fig. 5(d)).

Later in 2016, another model describes histamine receptor-mediated Ca^{2+} -dynamics that considers intercellular fluxes due to electrical and biochemical coupling between neighboring cells via gap junction. In this model, Ca^{2+} -dynamics is regulated by the $[Ca_c^{2+}]$ through multiple feedback loops, which includes the dynamics of PMCA, Ca^{2+} -channels, endoplasmic reticulum uptake, and IP_3 activity (Fig. 5(e)). In their investigation, endothelial cells were treated with various doses of histamine and the proposed model could successfully predict the dependence of ISI on histamine concentration. Similarly, a stochastic model for collective Ca^{2+} -signaling was developed that captures ATP-induced Ca^{2+} -oscillation [65]. This model considers the IP_3 -mediated opening of Ca^{2+} -channels, and the nonlinear dependence of the opening probability on the Ca^{2+} -concentration. The corresponding model structure is shown in Fig. 5(f). In order to incorporate the cell to cell variability, all the model parameters were varied to a specific fold change and the model could predict the pattern in a change in ISI of Ca^{2+} -oscillation in a cell population with a change in ATP concentration (Fig. 6(d)-(e)). The fraction of oscillating cells and the distribution of ISI at various doses agree well with experimental dose-response (Fig. 6(d)-(g)). In this study, a combination of modeling and experiment indicates that the frequency of Ca^{2+} -oscillation increases with agonist dose as well as cell density due to augmented connectivity between cells. Such mathematical models validated by the experimental data can further save on the cost of performing expensive imaging experiments. This can also allow us to understand the abnormalities (diseased conditions) and the effect of drugs, as the change can be reflected through a change in parameter values in the mathematical equations [25].

Most of the existing studies that combine experimental, as well as computational investigations on dose responses, are able to capture a qualitative similarity between the predicted and experimental responses. However, cell-specific modeling of Ca^{2+} -dynamics along with parameter estimation using an evolutionary algorithm can be crucial for the prediction of drug-dose response [25].

Another limitation of the models referred here is that they are based on the "well-stirred-reactor" assumption where all components of the signaling cascade are uniformly distributed in the cytoplasm. A state-of-art approach is to model the system as a reaction-diffusion process using partial differential equations and incorporation of stochastic

parameters that are less assumptive and accounts for the inherent variability in receptor states at different time points [77,78]. However, such an approach is computationally expensive. In the future, such models have the potential to generate stochastic cellular response and cell-to-cell variability and validate the dose-response curves at tissue levels.

5. Conclusions and perspectives

It has been noticed that a large amount of information is available on GPCR targeting agonists that are central in treating cardiac, respiratory and neuro-diseases, and activate G_{α_q} , G_{α_s} , and $G_{\alpha_{i/o}}$ pathways to regulate the Ca^{2+} -dynamics (Table 1 and Fig. 2). Most experimental investigations focus on understanding the role of agonists concentration in modulating $[Ca_c^{2+}]$ -oscillations. Parallel to this, there have also been efforts in understanding the role of GPCR targeting drugs in regulating Ca^{2+} -dynamics through constructing non-linear ODE models for $[Ca_c^{2+}]$ -oscillations (Table 2). Although some of the models describing the agonist-mediated encoding of $[Ca_c^{2+}]$ -oscillations are cell type-specific (Table 2) [54,59], many of the computational models describing the oscillation and the mechanisms are not specific to any cell line. Moreover, most of the computational models for Ca^{2+} -dynamics focus on G_{α_q} signaling and there are few investigations on G_{α_s} and $G_{\alpha_{i/o}}$ [54,79]. Existing models are able to decipher an interplay of positive and negative feedbacks present in the system which control uneven inter-spike-interval and frequency of $[Ca_c^{2+}]$ -oscillation. However, further studies are needed to be carried out to investigate the effect of specific drugs in a particular tissue type. Also, we need to have an integrated framework that investigates the dysregulation of Ca^{2+} in the context of various diseases.

The current paper presents a summary of G-protein coupled receptor-mediated models along with their network structures and feedback loops. Although the inclusion of all the models and mechanisms delineating GPCR mediated Ca^{2+} -dynamics is beyond the scope of this review, it is sincere efforts of the authors to provide a clear perspective of signal transduction with two examples in detail. This review is expected to provide in-depth insight on feedback loops and understanding of crucial kinetic parameters that are involved in regulating the specific features of $[Ca_c^{2+}]$ -oscillations including frequency and amplitudes. This review can be used as a guideline by pharmacologists and computational biologists for comprehending the systems perspectives underlying Ca^{2+} -signaling towards drug selection and screening of GPCR targeting drugs.

Author contribution

All authors contributed to the conception and design of the work. VD, SS and LG planned the work. VD, SG, RKG, and LG contributed to the writing of the manuscript. VD prepared the schematics and figures. KVV and LG reviewed the manuscript. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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