IP₃R, store-operated Ca²⁺ entry and neuronal Ca²⁺ homoeostasis in Drosophila

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Abstract
The IP₃R (inositol 1,4,5-trisphosphate receptor) releases Ca²⁺ from the ER (endoplasmic reticulum) store upon binding to its ligand Ins₃, which is thought to be generated by activation of certain membrane-bound G-protein-coupled receptors in Drosophila. Depletion of Ca²⁺ in the ER store also activates SOCE (store-operated Ca²⁺ entry) from the extracellular milieu across the plasma membrane, leading to a second rise in cytosolic Ca²⁺, which is then pumped back into the ER. The role of the IP₃R and SOCE in mediating Ca²⁺ homoeostasis in neurons, their requirement in neuronal function and effect on neuronal physiology and as a consequence behaviour, are reviewed in the present article.

Introduction
Changes in cellular Ca²⁺ concentration affect multiple signalling processes in both excitable and non-excitable cells [1]. Ca²⁺ signals can determine the nature and strength of neural connections in a circuit by specifying neurotransmitters and receptors [2]. The extracellular medium, as well as intracellular stores, act as sources and sinks for signalling Ca²⁺. Most neuronal Ca²⁺ signals have been attributed to entry of extracellular Ca²⁺ through voltage-operated channels or ionotropic receptors. Intracellular Ca²⁺ channels are also present in both vertebrate and invertebrate neurons [3], but their functions are less thoroughly understood. In Drosophila, it has recently been demonstrated that IP₃R (inositol 1,4,5-trisphosphate receptor)-mediated Ca²⁺ release and SOCE (store-operated Ca²⁺ entry) are both required in neurons for flight [4]. We have been studying the release of Ca²⁺ from intracellular stores through IP₃Rs and the role of Ins₃-mediated intracellular Ca²⁺ release followed by SOCE in primary cultures of Drosophila larval neurons. In the present review, we discuss how IP₃R-mediated and store-operated Ca²⁺ signalling functions in the maintenance of neuronal Ca²⁺ homoeostasis. These studies are relevant in the context of certain neurodegenerative diseases in vertebrates.

Signalling through Ca²⁺
Ca²⁺ exerts its effect through Ca²⁺-binding proteins, which in turn interact with proteins of different functions. High concentrations of cytosolic Ca²⁺ can precipitate phosphates and proteins. As Ca²⁺ cannot be metabolized or destroyed like other second messengers, the tight regulation of Ca²⁺ signalling is essential. Cells invest considerable energy to maintain a ~2000-fold Ca²⁺ barrier between the cytosol and either the ER (endoplasmic reticulum) store or the extracellular milieu. Resting cytosolic Ca²⁺ in most cells is ~100–400 nM, whereas that in the ER and extracellular milieu is approximately 2 mM [5]. Highly co-ordinated oscillations of cytosolic Ca²⁺ can dictate cellular function and responses, and there are dedicated components of the ‘Ca²⁺ signalling toolkit’ that control intracellular Ca²⁺ oscillations. These are mainly Ca²⁺ channels and pumps that sequester Ca²⁺ into intracellular and intercellular compartments. The level of intracellular Ca²⁺ at any given time in the cell is determined by a balance of processes that facilitate cytoplasmic Ca²⁺ elevation and those which extrude excess Ca²⁺ from the cytosol [6]. The ER and extracellular milieu are the primary source and sink of Ca²⁺, although there is also a contribution from mitochondria and lysosomal stores in metazoan cells.

Depending on their functions, cells employ different components from the Ca²⁺ signalling toolkit to generate and interpret a variety of Ca²⁺ signals [6]. These distinct combinations of the Ca²⁺ signalling toolkit impart a unique signature of Ca²⁺ homoeostasis that suit cell-specific physiology. External stimuli at the PM (plasma membrane) activate GPCRs (G-protein-coupled receptors), which in turn can activate a component of this Ca²⁺ toolkit, the IP₃R, which releases Ca²⁺ from ER stores. Reduced store Ca²⁺ triggers another component of the toolkit, store-operated Ca²⁺ channels, which replenish Ca²⁺ from the extracellular milieu to the cytosol. Molecular components of SOCE were discovered recently in RNAi (RNA interference) screens in Drosophila S2 cell lines [7–10]. STIM (stromal interaction molecule) is a single-pass transmembrane molecule in the ER membrane which has an EF hand in the ER lumen for sensing luminal Ca²⁺ depletion. On Ca²⁺ depletion from the ER, STIM starts forming clusters and shuttles close to the ER–PM junction where it activates Orai channels in the PM through which Ca²⁺ enters the cytosol [11]. The Ca²⁺ is pumped back into the ER store by the SERCA.

Key words: flight circuit, inositol 1,4,5-trisphosphate receptor (IP₃R), intracellular calcium, neurotransmitter, Orai, stromal interaction molecule (STIM).
Abbreviations used: ER, endoplasmic reticulum; IP₃R, inositol 1,4,5-trisphosphate receptor; PM, plasma membrane; SIA, spinocerebellar ataxia; SERCA, sarcoplasmic/endoplasmic reticulum ATPase; SOCE, store-operated Ca²⁺ entry; STIM, stromal interaction molecule.
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A model of existing and proposed pathways that contribute to spontaneous Ca\textsuperscript{2+} spikes and excitability in neurons

DAG, diacylglycerol; GPCR, G-protein-coupled receptor; IP\textsubscript{3}, inositol 1,4,5-trisphosphate; PIP\textsubscript{2}, phosphatidylinositol bisphosphate; TRP, transient receptor potential.

(sarcoplasmic/endoplasmic reticulum Ca\textsuperscript{2+} ATPase) pump. In vertebrates, there are three genes that encode isotypes of the IP\textsubscript{3}R, Orai and SERCA, whereas two genes encode isotypes of STIM. Different isotypes of these molecules in vertebrates have been found to effect cellular functions differentially [12–16]. The \textit{Drosophila} genome encodes just one copy of genes for the IP\textsubscript{3}R (\textit{itpr}), STIM (\textit{dSTIM}), Orai (\textit{dOrai}) and SERCA (\textit{dSERCA Ca-P60A}). Thus fruitflies serve as a useful genetic model for studying the role of these molecules in the systemic physiology of a whole organism.

**Requirement of IP\textsubscript{3}R function and SOCE in \textit{Drosophila} neurons for motor co-ordination and rhythmic flight**

Molecular genetic studies of \textit{itpr} mutants have revealed a critical role for the IP\textsubscript{3}R in larval viability and adult flight [17,18]. Interestingly, for both viability and flight, IP\textsubscript{3}R function has been mapped to neuronal subsets [aminergic and IPCs (insulin-producing cells)]. Moreover, the temporal requirement for the IP\textsubscript{3}R in the context of flight is primarily during pupal development [18,20]. Imaging Ca\textsuperscript{2+} in primary neuronal cultures derived from mutant \textit{itpr} third instar larvae revealed reduced Ca\textsuperscript{2+} release through the IP\textsubscript{3}R as well as compromised SOCE [4]. A dominant-negative allele of dSERCA (\textit{Kum\textsuperscript{170}}), dSTIM and dOrai suggests that Ins(1,4,5)P\textsubscript{3} signalling and SOCE function together in the context of flight circuit development and function. Moreover, flight defects in an \textit{itpr} mutant were suppressed to a greater extent on introducing both \textit{Kum\textsuperscript{170}} and \textit{Orai\textsuperscript{2}}. Neurons from these triple mutant (but flight-competent) animals continued to have reduced store Ca\textsuperscript{2+} concentrations, but exhibit robust SOCE. Increased Ca\textsuperscript{2+} release through mutant IP\textsubscript{3}Rs in the presence of a \textit{dOrai} hypermorphic allele suggests a feedback loop from Orai to IP\textsubscript{3}R function, which requires further investigation. These studies show for the first time that Ca\textsuperscript{2+} release through IP\textsubscript{3}R and SOCE play an important role in neuronal Ca\textsuperscript{2+} homoeostasis during development of neuronal circuits. How this signalling is tuned to the specification of neuronal function needs systematic elucidation, in terms of the precise neurons involved and their development and function.

**IP\textsubscript{3}R-mediated Ca\textsuperscript{2+} homoeostasis in neuronal excitability, neuromodulation and neural circuit formation**

The effect of altered Ca\textsuperscript{2+} homeostasis in neurons has been studied extensively in \textit{Xenopus}, where spontaneous Ca\textsuperscript{2+} spikes in the cytosol shape neuronal proliferation, migration and specification of activity by modulating the expression of voltage-gated ion channels and neurotransmitters. Altered expression of a neurotransmitter leads to an associated change in expression of neurotransmitter receptors in muscles at the neuromuscular junction and thus alters the architecture of the neural circuit, leading to behavioural changes [2]. The origin of these spontaneous Ca\textsuperscript{2+} spikes in neuronal excitability have been primarily attributed to
various channels and neurotransmitter receptors in the PM. Ionotropic glutamate receptors generate Na\(^+\) and Ca\(^{2+}\) currents that depolarize neurons throughout development, whereas TRP (transient receptor potential) channels are involved in the generation of spontaneous Ca\(^{2+}\) spikes in the growth cone [2]. A contribution from the ER store in generation of spontaneous cytosolic Ca\(^{2+}\) transients has also been demonstrated in Xenopus neurons, where application of caffeine could generate Ca\(^{2+}\) spikes with or without Ca\(^{2+}\) in the extracellular medium [21]. However, so far, none of these studies has implicated IP\(_3\),R-mediated Ca\(^{2+}\) release and SOCE in Ca\(^{2+}\) spike-induced neuronal excitability. Given the developmental requirement of IP\(_3\),R-mediated Ca\(^{2+}\) signals in initiating and maintaining flight rhythms, the dynamics of Ca\(^{2+}\) transients at different developmental time points in normal and flight-defective itpr mutants are of considerable interest. Altered Ca\(^{2+}\) homeostasis in itpr mutant neurons may lead to altered level and/or activity of voltage-gated and/or ligand-gated Ca\(^{2+}\) channels in the PM and expression of neuromodulators and/or neurotransmitters. Two functional neuronal domains identified in itpr mutants are the aminergic neurons and the insulin-like peptide-producing neurons, both of which are neuromodulatory. Neuromodulators play an important role in the function and formation of neural circuits such as central pattern generators for rhythmic and co-ordinated behaviour [22]. Thus changes in Ca\(^{2+}\) homeostasis in a neuromodulatory domain will first affect the cellular properties of modulatory neurons, and in turn this can lead to altered input to the central pattern generator which specifies the functioning of a motor circuit. In this context, flight-defective itpr mutants serve as a useful system to explore the neural mechanisms underlying a rhythmic motor behaviour such as insect flight, and the contribution of Ca\(^{2+}\) homeostasis and its role in neuromodulatory neurons to the function of the central pattern generator for flight.

The IP\(_3\),R is also important for human motor function since heterozygosity of IP\(_3\),R1 is the genetic basis for SCA (spinocerebellar ataxia) 15/16 [23]. Thus understanding the mechanism by which Ins(1,4,5)P\(_3\)-mediated Ca\(^{2+}\) release effects neuronal circuit function in the context of motor behaviour may also help interpret its cellular role in neurodegenerative diseases such as the SCAs that arise from distorted Ca\(^{2+}\) homeostasis [24]. Importantly, the suppression of defects due to IP\(_3\),R mutants by raising cellular SOCE in Drosophila, opens up new therapeutic possibilities for this class of neurodegenerative disorders.

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**References**


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