A gist of unheeded insect calcium channels as a target for insecticides

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A new class of insecticides, diamides provides exceptional control through action on a novel target, the ryanodine receptor (Cordova *et al.,* 2006). Diamides rank third (12%) in the global market after neonicotinoids (24%) and synthetic pyrethroids (15%) (Sparks *et al.,* 2020). There are five insecticides in the market registered against various group of insects belongs to lepidoptera, coleoptera, Hemiptera *etc*. Apart from these insecticides some diamide insecticides are in race includes cyhalodiamide, and tetrachlorantraniliprole and unnamed (Sparks *et al.,* 2020). A third class of diamides, "pyrrole-2 carboxamides" are currently under development (Cordova *et al.,* 2021). However, detailed examination of RyRs from field-collected or lab-selected resistant strains revealed mutations that affected residues located in the C-terminal transmembrane spanning domains is responsible for resistance development against diamides (Guo *et al.,* 2014). Development of insecticides with novel and unique modes of action is necessary to combat widespread insecticide resistance.

Calcium plays a vital role as a second important messenger in controlling physiological functions like neurotransmitter release, muscle contraction, hormone biosynthesis, development and metamorphosis, reproduction, sex pheromone synthesis, cold sensing, olfactory responses, diapauses, carbohydrate and lipid metabolism (Berridge *et al*., 2000; Toprak *et al*., 2021). Various kinds of calcium channels regulate these processes, *viz*., voltage-gated calcium channels (VGCC), Ryanodine and IP_3 sensitive calcium channels, store-operated calcium channels (SOCC), and Transient Receptor Potential (TRP) calcium channels (Luemmen, 2013). The reticence/ modification of these calcium channels leads to disparity in the calcium current, which disturbs the cells' physiological functioning. It ultimately leads to the death of an organism. To exploit calcium channels in pest management, it is vital to comprehend how they operate. Voltage-gated calcium channels and Ryanodine-sensitive calcium channels have been successfully used for pest management (Luemmen, 2013). Ryanodine-sensitive calcium channels are accompanied by ER (Endoplasmic reticulum) / SR (Sarcoplasmic reticulum) of neurons or muscle cells. Recently, two highly promising class of synthetic insecticides, diamides that disrupt calcium homeostasis by interfering with RyRs, have been introduced into the marketplace. VGCC is the macromolecular complexes that localize in the plasma membrane, open in response to membrane depolarization signal and mediate the movement of Ca^{+2} ions. Toxins of insects/ spiders are known to block VGCC reversibly (King *et al.,* 2008). Here is a short rundown of the physiology and pharmacology of various calcium channels that have not yet been targeted explicitly for insecticides.

Unheeded calcium channels for insecticide target

In addition to RyRC and VGCC, other calcium channels, including IP_3-sensitive calcium channels, TRP calcium channels, and store-operated calcium channels (SOCC), also play a crucial role in maintaining calcium homeostasis in insects (Luemmen, 2013). Since no synthetic insecticides are currently available that target these channels, it is imperative to comprehend their structure and pharmacology.

1. IP_3 Sensitive calcium channels

IP_3-sensitive calcium channel is the one which is activated by the secondary messenger inositol 1,4,5-trisphosphate (IP_3) and Ca^{2+} and regulates the intracellular calcium level and is localized on the ER membrane. The IP_3R and RyR are members of a family of tetrameric intracellular Ca^{2+}-release channels encoded by single genes in insects. Insect IP_3Rs are commonly composed of three regions: the ligand binding region (Ca sensor domain), regulatory and transducing region (coupling domain), and channel forming domain. The ligand binding region consists of three subdomains *viz*., SD (suppressor domain), IBC-β (IP_3 binding core- β) and IBC-α (IP_3 binding core- α) (Fig. 1) (Toprak *et al*., 2021). There is evidence that ligand binding sites influence the function of the channel-containing domain in both receptors.

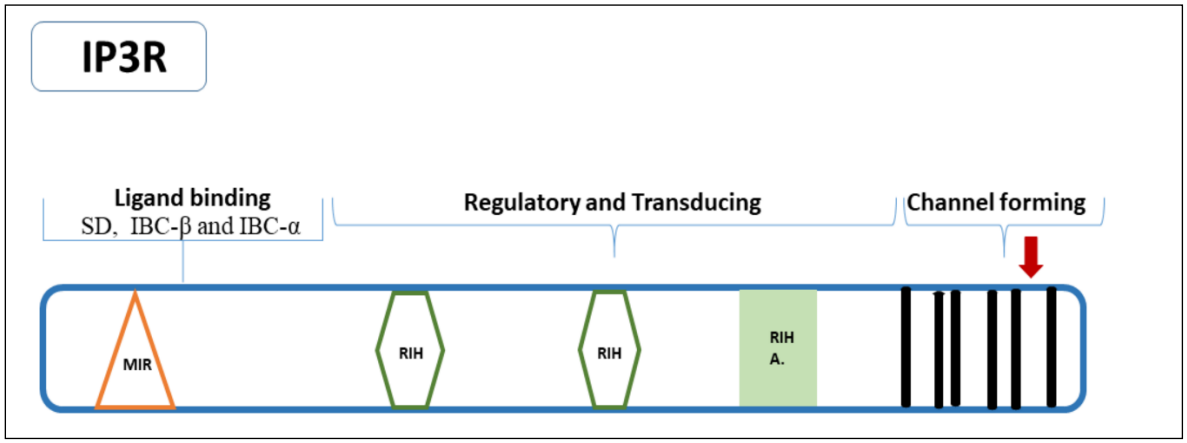

Figure 1: Structure of IP_3Rs (Source: Toprak *et al*., 2021)

Pathway of IP_3Rs: IP_3Rs are expressed in most cells, particularly in the ER of neurons, fat body adipocytes, and oocytes. Low cytoplasmic Ca^{2+} activates IP_3R, while high concentrations inhibit the channel's activity. G-protein-coupled receptors (GPCRs) in the plasma membrane of the cell stimulate phospholipase C (PLC) that hydrolyzes the phosphorylated plasma membrane glycolipid, phosphatidylinositol 4,5-bisphosphate (PIP_2), into secondary messengers diacylglycerol (DAG) and IP_3

(Fig. 2). IP_3 binds to IP_3-binding sites in the N-terminus of the tetrameric IP_3R to initiate conformational changes that are transmitted down to the transmembrane region leading to the opening of the Ca^{2+}-permeable pore away from the IBC to release the Ca^{2+} from the ER (Toprak *et al*., 2021).

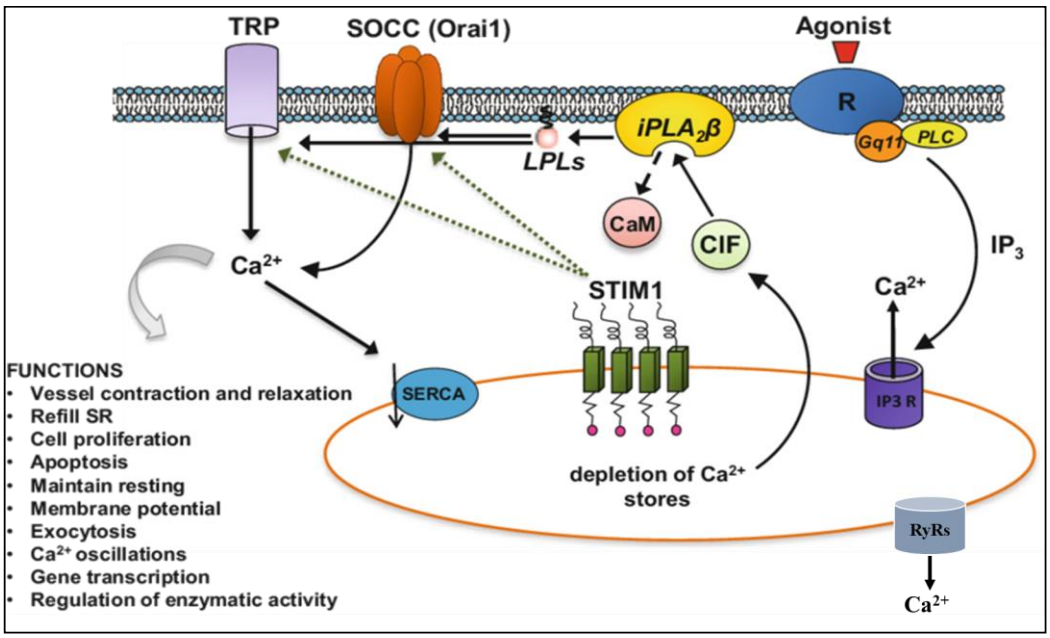

Figure 2: Different Calcium Channels (Modified from the image of Smani et al., 2016)

Markedly, IP_3Rs are essential for viability, but no insecticidal compounds targeting IP_3Rs have been reported in the literature (Luemmen, 2013). The development of pesticides that interfere with the receptors of IP_3 has great potential in pest management.

2. TRP calcium channels

A specific type of calcium entry channel in the plasma membrane was initially identified in a *Drosophila* mutant defective in the visual system. TRP (Transient Receptor Potential) channels translate a variety of visual, mechanical, and chemical stimuli into neuronal signals, thereby serving key functions in sensory processes. TRP channels have evolved to considerable diversity in insects, as indicated by the fact that the *Drosophila* genome contains members of seven TRP subfamilies. Based on amino acid sequence homology, the TRP channel superfamily is classified into seven related subfamilies: TRPC, TRPM, TRPV, TRPA, TRPP, TRPML, and TRPN. TRP channels possess six predicted transmembrane helices and a putative pore loop connecting helices 5 and 6. TRP channels lack the positively charged voltage-sensor residues in helix 4, a characteristic structural feature of voltage-gated Na, K, and Ca^{+2} channels (Fig. 2) (Phillips *et al.*, 1992; Luemmen, 2013).

Some TRP channel subtypes, like the mammalian $TRPV_1$, are activated by capsaicin, vanilloids, and a variety of potentially noxious or irritating compounds (Kissin and Szallasi, 2011). The insecticidal activity of horseradish or yellow mustard extracts was attributed to the presence of allyl- and benzyl-isothiocyanates. The finding that those compounds activated $TRPA_1$ channels in vitro led to the proposal that TRP channels may be possible target sites for new insecticides (Nagata, 2007). Interestingly, some insecticides have been developed to target chordotonal organ TRPV channels (Pyridine azomethine derivatives). However, still there is a wide scope for developing synthetic insecticidal compounds interfering with TRP calcium channels.

3. Store Operated Calcium Channels (SOCC)

Depleting intracellular calcium stores through the activity of calcium release channels triggers calcium entry from the extracellular environment. The process is termed store-operated calcium entry. It is mediated by specialized calcium-conducting channels in the plasma membrane called SOCC/ CRAC (Ca^{2+} release-activated calcium channels). Initially, members of the TRP channel superfamily had been implicated in SOCE. Later, a genome-wide RNAi screen in *Drosophila* for genes affecting SOCE revealed several genes influencing SOCE.

Pathway of SOCC: The stromal interaction molecule (STIM)-Orai1 complex are major player involved in SOCE (Fig. 2). STIM is normally located in the ER transmembrane and senses luminal Ca^{2+} depletion, which leads to its translocation to junctions between the ER and plasma membrane, where it couples with the plasma membrane Ca^{2+} channel protein Orai1. This coupling activates Ca^{2+} release-activated Ca^{2+} (CRAC) channels in the plasma membrane, allowing Ca^{2+} influx from the extracellular pools to the cytosol and then from the cytosol to the ER through SERCA (Sarco/endoplasmic reticulum Ca^{2+} ATPase). Elevation of cytosolic Ca^{2+} to certain levels inactivates CRAC channels, thereby terminating Ca^{2+} influx into the cell, a process known as Ca^{2+}-dependent inactivation (CDI). It is noteworthy that the primary Ca^{2+}-binding protein, calmodulin, is involved in CDI by binding to STIM, disrupting the STIM-Orai1 complex (Luemmen, 2013).

In insects, however, little is known about the pharmacology of SOCC, and potential lead structures for insecticide discovery are not known. As intracellular calcium level is important for cell activity, the development of insecticides that interfere with the store-operated calcium entry process could become a potential insecticide in pest management.

Conclusion

The different calcium-conducting ion channels regulate various physiological processes such as neurotransmission, muscle contraction, and lipid metabolism. Accordingly, calcium channels and pumps have been proposed as novel insecticide target sites. Yet, only two calcium channels, RyRC and VGCC, are exploited for pest control. There is a broad scope for investigating other channels like IP_3 sensitive calcium channels, TRP calcium channels and store operated calcium channels. Further, calcium channels involved in other physiological processes, such as JH synthesis, ecdysone synthesis, lipid metabolism, fluid transport *etc*., are not yet characterized for insecticide targets. It has been expounded that calcium channels comprise promising pesticide targets to meet the requirements of future pest management.

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