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Ca²⁺ Signaling: An Outlook on the Characterization of Ca²⁺ Channels and Their Importance in Cellular Functions

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Abstract

Calcium (Ca²⁺) is essential in regulating a plethora of cellular functions that includes cell proliferation and differentiation, axonal guidance and cell migration, neuro/enzyme secretion and exocytosis, development/maintenance of neural circuits, cell death and many more. Since Ca²⁺ regulates so many fundamental processes, it could be anticipated that numerous Ca²⁺ channels and transporters will assist in regulating Ca²⁺ entry across the plasma membrane. Towards this several Ca²⁺ channels such as voltage-gated channels, store-operated Ca²⁺ entry (SOCE) channels, NMDA, AMPA and other ligand gated channels have been identified. In recent years research focus has been targeted towards identification of the precise function of these essential channels. Furthermore, characterization of these individual Ca²⁺ channels has also gained much attention, since specific Ca²⁺ channels have been shown to influence a particular cellular response. Moreover, perturbations in these Ca²⁺ channels have also been implicated in a spectrum of pathological conditions. Hence, understanding the precise involvement of these Ca²⁺ channels in disease conditions would presumably unveil avenues for plausible therapeutic interventions. We thus review the role of Ca²⁺ signaling in select disease conditions and also provide experimental evidence as how they can be characterized in a given cell.

Introduction

Ca²⁺ Signaling

Most of us see the word Ca²⁺ daily, whether it is read on the label of a multivitamin or seen on a carton of milk, but do not realize the complexity and vast array of functions that this simple divalent cation plays on a molecular level. The majority of people know Ca²⁺ is involved in bone development and in the prevention of diseases such as osteoporosis; however, Ca²⁺ is one of the most abundant signaling molecules found in the human body which regulate functions ranging from the cell cycle and embryogenesis to cell death. Disruptions in Ca²⁺ signaling has been linked to the pathogenesis of numerous diseases such as, but not limited to Huntington's disease, Alzheimer's disease, Cancer, Congenital Heart Failure, and Diabetes. The focus of this chapter is to give an appreciation for Ca²⁺ signaling, characterization of Ca²⁺ channel activity, and how certain diseases arise due to disruptions or remodeling of the Ca²⁺ signaling cascade.

Many cellular responses act like factory machines, and their efficiency depends on a delicate balance between the input and output signal. The same can be said for Ca²⁺ signaling where the concentration of Ca²⁺ acts as the signal. One of the most important parts of Ca²⁺

signaling is the cell's ability to regulate this signal, since cells use the concentration of Ca^{2+} as a mechanism to drive many cellular processes. In order for a cell to elicit a cellular response due to Ca^{2+} signaling, it must be able to regulate the concentration of Ca^{2+} in different cellular locations. In any given cell, Ca^{2+} concentrations can range from its basal cytosolic concentration of 100 nM to as much as 1–10 μM when the cell is ready to produce a signaling cascade [1]. Importantly, certain cellular responses have an optimum Ca^{2+} concentration which once reached, signaling proteins can create a signal cascade which act on downstream effectors to activate transcription factors or other proteins to aid in the regulation of that response (Fig. 6.1). Before the cell is able to elicit a Ca^{2+} signal to activate certain processes required to maintain a healthy cell, it must be able to sustain a steady level of Ca^{2+} within its stores and in the cytoplasm. Since cells and their corresponding responses are sensitive to varying levels of Ca^{2+} , they must create a mechanism to keep Ca^{2+} at its basal cytosolic concentration except to elicit a cellular response. Thus, cells have developed a sophisticated mechanism that balances the Ca^{2+} levels, by several methods that include compartmentalization, chelation, or expulsion of Ca^{2+} from the cell (Fig. 6.1) [1].

Ca^{2+} Influx Channels and Cellular Homeostasis

The plasma membrane and endoplasmic reticulum are two of the most basic barriers for the compartmentalization of Ca^{2+} . The cell adapts Ca^{2+} channels to aid in the compartmentalization, expulsion, and transport of Ca^{2+} (Fig. 6.1) [1]. The plasma membrane acts as a divider to keep intracellular and extracellular Ca^{2+} concentrations separate. ATPase pumps on the plasma membrane known as plasma membrane Ca^{2+} ATPases (PMCA pumps) push Ca^{2+} out of the cell against its concentration gradient at the expense of ATP^2 . Other proteins such as $\text{Na}^+/\text{Ca}^{2+}$ (NCX) and $\text{Na}^+/\text{Ca}^{2+}$ - K^+ (NCKX) exchangers use the concentration gradient of other ions such as sodium to extrude one Ca^{2+} ion for three sodium ions or cotransport one Ca^{2+} and potassium ion to the outside of the cell for four sodium ions into the cell, respectively [2]. The endoplasmic reticulum is another organelle that acts as a barrier to aid in the separation of concentration. The endoplasmic reticulum can also act as a storage unit for Ca^{2+} to be used later when the cell needs to elicit a Ca^{2+} dependent response. ATPases on the endoplasmic reticulum known as sarcoendoplasmic reticular Ca^{2+} ATPases (SERCA pumps) push Ca^{2+} into the endoplasmic reticulum at the expense of ATP^2 . Due to the actions of the plasma membrane, endoplasmic reticulum, and their corresponding Ca^{2+} channels, the cell is able to transport Ca^{2+} into the extracellular space or into the endoplasmic reticulum for storage to be released in response to certain agonist binding which will be discussed later.

Ca^{2+} expulsion from the cell is only applicable when dealing with large increases in the intracellular concentration of Ca^{2+} . As mentioned earlier, Ca^{2+} signaling occurs in response to changes in Ca^{2+} concentration ranging from minute to large increases in intracellular Ca^{2+} . Cells are able to regulate the amount of Ca^{2+} entering the cell much the same way they control the amount of Ca^{2+} leaving the cell. Ca^{2+} channels such as voltage-gated Ca^{2+} selective channels, store/receptor operated Ca^{2+} entry channels, and NMDA/AMPA channels coordinate the amount of Ca^{2+} entering the cell at any given time (Fig. 6.1).

Voltage-Gated Ca^{2+} Channels—Voltage-gated Ca^{2+} selective channels (CaV) are found on the plasma membrane where they function in Ca^{2+} entry elevating the intracellular concentration of Ca^{2+} . These channels function by predominantly using the electrochemical gradient that is created by the separation of charges between the intracellular and extracellular space thus creating a polarized cell [3]. The channel opens due to a helix-turn-helix loop containing positively charged amino acids that is able to sense voltage changes allowing Ca^{2+} to enter the cell [1]. Voltage-gated Ca^{2+} selective channels are able to create vast increases in intracellular Ca^{2+} concentrations in a matter of milliseconds making these

types of channels the fastest and most efficient channel to elicit a Ca^{2+} signaling response [3]. Since the regulation of the amount of Ca^{2+} entering and exiting the cell is controlled by plasma membrane channels; the cell is able to create an efficient mechanism for maintaining Ca^{2+} -dependent processes.

Store/Receptor-Operated Ca^{2+} Channels—Store-operated Ca^{2+} entry (SOCE) is a unique mechanism for Ca^{2+} entry, since it involves many channels and proteins throughout the cell, which is unlike voltage-gated channels which rely solely on the separation of charges between the intracellular and extracellular space. Ca^{2+} in the ER is continuously leaking out into the cytosol (due to concentration gradient), which is constantly pumped back into the ER by the high activity of SERCA pumps and avoids emptying of the ER Ca^{2+} stores. However, upon agonist stimulation second messengers are generated that eventually deplete ER Ca^{2+} that relays the signal to the plasma membrane and initiate Ca^{2+} entry via the SOCE mechanism [4]. One of the long lasting question in the field was as how does a cell respond when these pumps are blocked or the cytosol doesn't have enough Ca^{2+} to sustain the repletion of ER by SERCA pumps? The answer to the question came by the identification of the SOCE, which satisfies this requirement by acting as a response mechanism through crosstalk between the ER and Ca^{2+} channels on the plasma membrane, thereby allowing Ca^{2+} to enter the cell from the extracellular space and refill the Ca^{2+} stores in the ER [4].

Changes in ER $[\text{Ca}^{2+}]$ are sensed through a single transmembrane ER Ca^{2+} sensing protein known as stromal interacting molecule 1 (STIM1), via an EF hand which is located on the luminal C-terminal tail [4]. Upon ER Ca^{2+} store depletion, Ca^{2+} dissociates from the EF hand causing STIM1 to aggregate on the ER through the assistance of its cytosolic N terminus sterile α -motif forming puncta near the plasma membrane [5]. These puncta translocate close to the plasma membrane where it interacts with a four transmembrane spanning plasma membrane protein, Orai or TRPCs or both, to allow Ca^{2+} to enter the cell and refill the ER stores. Although recent research has put forth a plausible working model, several issues such as how STIM1 is regulated and how it is translocated to the plasma membrane still remains a mystery today. Thus, further scientific inquiries into the regulation of STIM1 can hopefully yield interesting results that will open further areas of research in the field of Ca^{2+} signaling.

SOCE function is not only limited to refilling of the intracellular Ca^{2+} stores, but can elicit a Ca^{2+} response itself propagating the signal downstream to its effectors and yielding a specific cellular action. It can accomplish this by using the phosphatidylinositol signaling pathway that is also known as receptor-operated Ca^{2+} entry. When an agonist binds to a G protein-coupled receptor (GPCR) causing hydrolysis of the receptors subunits, $G\alpha$ and $G\beta/\gamma$, $G\alpha$ moves downstream to activate phospholipase C (PLC) which in turn cleaves phosphatidylinositol 4,5 biphosphate (PIP₂) into 1,4,5 inositol triphosphate (IP₃) and diacylglycerol (DAG) [6]. IP₃ is able to bind to an ER receptor named 1, 4, 5 inositol triphosphate receptors (IP₃Rs) where it causes a conformational change in the receptor allowing Ca^{2+} to exit the ER stores and raise the cytosolic Ca^{2+} ($[\text{Ca}^{2+}]_i$) from its basal level of 100 nM to as much as 1 μM in a matter of seconds priming the cell for a Ca^{2+} dependent cellular response [6].

How does the cell recognize an elevation in Ca^{2+} concentration? How is the cell able to translate the increase in $[\text{Ca}^{2+}]$ to a signal for downstream proteins to activate a cellular process such as apoptosis or transcription? A cell's answer to these questions is the creation of Ca^{2+} binding protein. Many proteins within the cell contain Ca^{2+} binding domains such as calmodulin whose EF hand, like the one seen in STIM1, is able to bind Ca^{2+} to regulate cellular functions [4]. Upon Ca^{2+} binding, calmodulin is able to undergo a conformational

change which causes inhibitory proteins to dissociate from calmodulin making it accessible to interact with other proteins. The conformational change that has occurred upon Ca^{2+} binding allows active sites to emerge that have previously been hidden by the closed conformation [7]. The Ca^{2+} bound calmodulin has numerous roles in the cell. One such role is the ability to aid in certain phosphorylation pathways such as the calmodulin kinase family (CAMK). Once Ca^{2+} bound calmodulin binds to calmodulin kinase, autoinhibitory proteins dissociate allowing CAMK to experience autophosphorylation. Autophosphorylation allows kinase activity to be active for a longer duration [8] and this extended kinase activity exerts its effect on many downstream proteins which participate in numerous cellular processes. In addition, Ca^{2+} binding proteins are also able to propagate the Ca^{2+} signal to a wide array of different proteins found in different areas of the cell which are responsible for triggering precise cell specific tasks.

Characterization of Ca^{2+} Channels

Measurement of Ca^{2+} Currents—Patch clamping is a technology that is widely used to record multiple ion currents including Ca^{2+} currents. Using a glass microelectrode it is possible to record Ca^{2+} channel activity, since it seals the surface of cells by over $10^{10} \Omega$ resistance, thereby electrically isolating this tip-touched small region on the plasma membrane from its vicinity, thus maintaining membrane potential to monitor and record the single- and whole cell-channel Ca^{2+} currents [9].

SOCE Currents—SOCE currents are generally pretty small, for example, in HEK293 cells and in HSG (human submandibular gland) I_{SOCE} are only 0.5pA/pF and 2pA/pF respectively. In addition, the current and voltage (IV) relationship of I_{SOCE} is slightly different in different cell types. For example, in HSG and RBL cells, the IV curve exhibit inward rectification (however the amount of inward rectification is different in both cells). Meanwhile in HSY cells, the IV curve is close to linear, suggesting that different channels could contribute to the same current [10]. In addition, the reversal potential of I_{SOCE} also varies in different cells and is close to 0 or +40 mV in most cell types (Fig. 6.2).

Measurement of SOCE Currents—For the recording of I_{SOCE} , the basic protocol is as follows: In standard whole cell mode, every 4 s, a voltage ramp is applied that ranges from -90 to 90 mV over a period of 1 s with a holding potential of 0 mV. Using this protocol, it's easy to monitor the development of I_{SOCE} over time as well as establishing the IV relationship of the I_{SOCE} . The external solution used is the modified Ringer's solution with the exception of using a high concentration of Ca^{2+} . Internal solution consists of Cs^+ along with Ca^{2+} chelators. Importantly, I_{SOCE} can be activated by several ways, which includes: (i) Intracellular dialysis with high concentration of the chelators (10 mM EGTA, or 10 mM BAPTA) that can activate an inward Ca^{2+} current after a short delay. (ii) In contrast, addition of IP_3 along with Ca^{2+} chelators in the internal solution develops a current, which is relatively fast. (iii) SERCA pump inhibitors such as thapsigargin or ionomycin can also deplete Ca^{2+} stores in order to activate the I_{SOCE} currents (Fig. 6.2).

Facilitation of SOCE—In most cell types, I_{SOCE} is so small that it is important to enlarge the current amplitude by several ways. Ca^{2+} has a positive effect on I_{SOCE} , since its potentiation depends on external Ca^{2+} concentrations [11]. Thus, increasing Ca^{2+} concentration of the external solution (10–20 mM) is generally sufficient to experimentally record I_{SOCE} . However, Ca^{2+} regulation of I_{SOCE} is complex and besides having a positive effect, it is also involved in Ca^{2+} -dependent inactivation, which is further characterized by either fast or slow inactivation [12, 13]. Thus, exposing the cells to a normal solution can minimize the period of cells that are exposed to high Ca^{2+} concentration before initiating patch clamping experiment is important. Another way to reduce the inactivation is to use

Ca^{2+} chelators. EGTA has been shown to be less effective on fast inactivation, whereas BAPTA is a more rapid chelator and reduces fast inactivation. In addition, to increase the conductance of I_{soc} it is also advisable to use DVF (divalent cation-free) solution, where removal of all extracellular divalent cation, results in a large Na^+ currents through SOCE channels.

Voltage Dependent Ca^{2+} Currents—Voltage dependent Ca^{2+} currents (VDCC) are present especially in excitable cells. For recording VDCC, K^+ current have to be blocked by the addition of Cs^+ or by applying 4-aminopyridine. Similarly, Na^+ is also blocked by either replacing Na^+ from the solution or by adding tetrodotoxin in the solution. In whole cell mode, current is elicited from a holding potential of -70 mV by 150-ms depolarizing voltage step that range between -70 and $+70$ mV. A typical VDCC and IV relationship in hippocampus cells is shown in Fig. 6.2. There is a remarkable rundown effect, which will affect recording of the VDCC and including 10 mM ATP intracellularly can decrease the rundown effect. However, the best solution for abolishing rundown of VDCC is using perforated patch recordings, with nystatin (100 ug/ml) or amphotericin B (30 ug/ml) in the intracellular solution. These polyene antibiotics form pores to allow small ion to permeate in a cell-attached patch mode, thereby abolishing rundown by preventing the leak. However, a potential problem for perforated path recording is that sometimes the access resistance is large that will decrease the amplitude of VDCC.

Ca^{2+} Activated K^+ Currents— Ca^{2+} activated K^+ current is another way to monitor the changes in Ca^{2+} concentrations especially in the subplasma membrane region for its tight regulation by Ca^{2+} concentration [14]. To record these currents in a whole cell configuration, gap-free mode is used with a holding potential of 0 mV, and addition of Ca^{2+} activators (such as IP_3 , Carbachol) can induce K_{Ca} that are much larger and are easy to record. For recording the IV relationship, the membrane potential is changed from -120 to 80 mV using a 20 mV step protocol.

Ca^{2+} Channels and Their Implication in Various Diseases

To the human eye, diseases present themselves as a collection of signs and symptoms varying in the degree of severity. For instance, Alzheimer's disease can only be viewed by the symptoms it presents such as a progressive decline in cognitive function. This cognitive dysfunction manifests itself as memory loss, trouble understanding visual and spatial relationships, and a decrease in judgment. However, on a molecular scale, diseases arise due to cellular dysfunctions and different diseases correlate to different abnormalities in cellular functions. Cellular abnormalities can vary from the level of transcription to protein localization and regulation. Thus, the rest of this chapter is going to be focused on cellular dysfunctions and its correlation to the progression of different diseases with respect to Ca^{2+} signaling. As mentioned previously, Ca^{2+} play a vital role in numerous cell functions; therefore, the diseases that arise from disruptions in Ca^{2+} signaling range from neurodegenerative diseases such as Huntington's disease to cardiomyopathies such as congestive heart failure.

Ca^{2+} Channels in Alzheimer's Disease—A disruption in Ca^{2+} signaling is not known to be the cause of Alzheimer's disease (AD). On the other hand, there is growing evidence supporting the hypothesis that a remodeling of the Ca^{2+} signaling is involved in the slow decline in cognitive thinking seen in AD. Within the scientific community, AD has been studied extensively leading to the discovery of amyloid fibrils and plaques which aggregate outside of neurons [15]. The cause of this buildup in amyloid plaques is the hydrolysis of β -amyloid precursor protein (APP) by the β - and γ -secretase complex. The product of the hydrolysis, β -amyloid protein ($\text{A}\beta$ protein), tends to polymerize into the plaques and fibrils

seen in the pathology of Alzheimer's disease [16]. Although many factors can play a role in the pathogenesis of AD, all factors essentially affect this amyloid cascade hypothesis leading to abnormal amounts of amyloid production. There are two forms of Alzheimer's disease, sporadic and familial, that have been studied. However, both of these disease forms affect the amyloid cascade hypothesis and the only difference lies between the two is as how the hypothesis is affected. Sporadic AD is the most widely known as it is this form which is the most common among people who develop AD. Sporadic AD is generally characterized by a slow severe decline in cognitive functions [17]. On the other hand, familial Alzheimer's disease (FAD) is developmentally a faster form than sporadic AD as it involves mutations in the amyloid pathway. Some examples of the more commonly mutated proteins that are involved in FAD are APP, members of the presenilin family, and apolipoproteins [17]. Sporadic and familial Alzheimer's disease are under extensive scientific studies trying to elucidate the link between the development of AD and the neuronal cell abnormalities and degeneration leading to cognitive dysfunction.

There are many current hypotheses proposing the involvement of Ca^{2+} in Alzheimer's disease. Most of these hypotheses involve the amyloidogenic pathway remodeling the Ca^{2+} signal in cells exhibiting amyloid plaques and fibrils. One such role includes α -secretase cleavage of the membrane bound APP into a soluble portion sAPP α and the C terminus membrane bound portion (CTF α). β -secretase is able to hydrolyze (CTF α) into an APP intracellular domain (AICD), which translocates to the nucleus and act as a transcription factor and may have an effect on the expression of ryanodine, a receptor on the SR that when activated releases Ca^{2+} from the SR stores, and calbindin proteins [16, 18]. It has been shown that individuals with AD have a down regulation of the expression of the Ca^{2+} buffering protein calbindin which aids in restricting the Ca^{2+} amplitude thus regulating Ca^{2+} signaling [16]. Another important hypothesis that is being studied suggests that a specific amyloid plaque, $\text{A}\alpha_{42}$, may act as a ligand for the membrane bound cellular prion protein receptor, which when activated may carry out Ca^{2+} cellular responses such as activating NMDA receptors allowing Ca^{2+} into the cell that induces excitotoxicity [19]. Taken together, these current hypotheses drastically change the overall levels of $[\text{Ca}^{2+}]_i$ throughout the cell leading to a remodeling of the Ca^{2+} signal that can activate processes, which are involved in neuronal cell death (such as caspases or cytochrome C) to assist in the neurodegenerative damages present in AD.

The role of Ca^{2+} in Alzheimer's disease also extends its reach into the symptomatic manifestations of learning and memory deficiencies. Memories are either stored or erased according to varying levels of Ca^{2+} which effects the phosphorylation and trafficking of the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic receptors (AMPA) [20]. Long-term potentiation (LTP) occurs at high $[\text{Ca}^{2+}]_i$, which can signal the phosphorylation and membrane insertion of the AMPA receptor, thereby, allowing memories to be stored for a short term [21]. On the other hand, long-term depression (LTD) occurs at lower $[\text{Ca}^{2+}]_i$ which allows activation of phosphatases such as Ca^{2+} -dependent calcineurins to remove phosphates from the AMPA receptor, thus, removing it from the plasma membrane by the process of endocytosis [22]. LTD leads to the elimination of temporary memories previously stored by LTP. Thus, overall, LTP and LTD occur at varying Ca^{2+} levels and are associated with different times throughout the day. LTP occurs while learning during the day leading to a quick spike in Ca^{2+} , while LTD occurs during sleep when a low steady level of Ca^{2+} is maintained [21]. In contrast, permanent memory storage occurs during different stages of sleep when LTP and permanent memory storage overlap, it is when that the brain transfers memories from short term to storage for long term, however, any memories that did not overlap during this transition are erased by LTD. Interestingly, in AD, remodeling of the Ca^{2+} signal causes changes in $[\text{Ca}^{2+}]_i$ which can affect memory storage by the LTP and LTD mechanisms. One possible explanation for memory loss seen in AD is the change in

Ca^{2+} levels that leads to the continuous activation of LTD, thereby causing memories stored by LTP to be constantly erased before the transfer to permanent storage can begin [21].

Ca^{2+} Channels in Huntington's Disease—Huntington's disease (HD) is an autosomal dominant neurodegenerative disease characterized by disruptions in motor skills and cognitive functions such as the development of chorea and dementia. On a molecular level, HD appears due to a trinucleotide expansion, CAG, in the gene encoding for a 350 kDa cytosolic protein [23]. In HD, the expanded CAG repeats enable the protein to be translated with an expanded polyglutamine (polyQ) tract on its amino terminus. The length of the polyQ tract reveals an inverse relationship between the age of onset of the disease and the number of glutamines present in the amino terminus [24]. Symptomatic manifestations of HD most commonly occur with individuals presenting at least a 35Q stretch in huntingtin gene (Htt) [25]. Individuals with the mutated form of Htt start to exhibit symptoms later in life, most commonly become symptomatic anywhere from 35 to 45. However, symptoms may arise earlier in life depending on the severity of the polyQ tracts [26]. Mutated Htt may play several roles in the disruption of numerous cellular functions such as axonal transport, proteasomes, and endocytosis [26]. In addition, Ca^{2+} signaling involving the NMDA receptor, mitochondrial Ca^{2+} homeostasis, and the regulation of the inositol (1,4,5)-triphosphate receptor (InsP_3R) may also play a role in HD [27].

NMDAR is one of the three classes of ionotropic glutamate receptors that lead to a drastic increase in Ca^{2+} influx upon the activation of NMDAR. A possible outcome of the over activation of NMDAR is Ca^{2+} overload leading to cell death in these neurons. Experiments have been developed to ascertain whether there is a causal role in the activation of NMDAR and neuronal cell death as seen in HD. In one of the classical experiment overexpression of the expanded Htt (Htt-138Q), but not the wild type Htt (Htt-15Q) was able to activate NMDAR [28]. A possible role in expanded Htt activating NMDAR was then established which was mediated through its interaction with PSD95. Htt is able to bind to PSD95, a modular adaptor protein; however, expanded Htt has been shown to have a decreased interaction with PSD95 [29]. PSD95 is known to associate with NR2 subunits which assist in the recruitment of Src tyrosine kinase that phosphorylates NMDAR receptors leading to an increase in NMDAR activation [30]. Due to the decreased association between expanded Htt and PSD95, PSD95 interaction with NR2 increases leading to Src kinase to phosphorylate NMDAR.

IP_3R is intimately involved in the release of Ca^{2+} from the ER stores (as mentioned earlier), but how does this receptor play a role in neuronal cell death seen in HD is a new concept? Several investigators have studied the relationship between Htt and IP_3R . It has been shown that expanded Htt is able to sensitize $\text{IP}_3\text{R}1$, the neuronal isoform of IP_3R , to IP_3 due to a tertiary interaction with Htt-associated protein 1A (HAP1A) [31]. Further evidence has been discovered indicating Htt-138Q, but not the normal length Htt-23Q, leading to the sensitization of IP_3R , thereby increasing cytosolic Ca^{2+} levels [31]. Taken together, the evidence indicate that increased cytosolic Ca^{2+} concentration due to the sensitization of IP_3R and activation of NMDAR could lead to cytosolic and mitochondrial Ca^{2+} overload causing neuronal death as observed in HD.

Expanded Htt is also able to interfere in many pathways involving Ca^{2+} signaling leading to progressive decline in cellular function due to Ca^{2+} mishandling. Proapoptotic pathways are sensitive to changes in Ca^{2+} in the mitochondria and cytosol. A raise in cytosolic Ca^{2+} , due to activation of NMDAR and IP_3R by expanded Htt, can lead to the activation of a family of proapoptotic proteins such as Bcl-2 [32]. During times of Ca^{2+} overload, the mitochondria can act as a Ca^{2+} sink relieving the cytosol of Ca^{2+} . If the mitochondrial Ca^{2+} increases to the point where the mitochondria can no longer withstand the Ca^{2+} overload, the

permeability transition pore (PTP) in the mitochondria opens, thereby releasing Ca^{2+} and other proapoptotic factors such as cytochrome C [33]. Ca^{2+} mishandling as seen in HD is an extensively researched area in the scientific community, and through this research, novel drugs may be developed to regulate the amount of Ca^{2+} entering the cell or exiting the ER. Thus, novel compounds could be developed that can alleviate neuronal cell death by HD.

Ca^{2+} Channels in Congestive Heart Failure—Congestive heart failure is a condition characterized by a slow progression of fatigue and breathlessness that eventually leads to death of an individual. The cause of these symptoms is a heart defect in which the heart cannot provide the body's tissue with a sufficient amount of blood. Insufficient blood supply to body tissue triggers a cascade of events such as neurohormone stimulation and intracellular signaling to compensate for the loss of cardiac performance. However, as the disease progresses, these events act in concert leading to complete organ failure. Disruptions in Ca^{2+} handling are a major factor in the progression of congestive heart failure. A heart contraction begins due an action potential caused mainly by an influx of Na^+ ion to depolarize the sarcolemma. The depolarization of the membrane activates L-type Ca^{2+} channels, which increases Ca^{2+} entry. Furthermore, this increased cytosolic Ca^{2+} also allows Ca^{2+} to be extruded from the sarcoplasmic reticulum (SR) through Ca^{2+} -induced Ca^{2+} release mechanism, thereby further raising the cytosolic $[\text{Ca}^{2+}]_i$ levels [34]. An increase in cytosolic $[\text{Ca}^{2+}]_i$ allows Ca^{2+} to bind to troponin C, a myofilament protein, that then leads to muscle contraction. Heart relaxation occurs by the activation of SERCA pumps, sarcolemma Na^+ - Ca^{2+} exchangers (NCX), sarcolemma Ca^{2+} ATPases, and mitochondrial Ca^{2+} uniporters [35]. These channels act together to decrease the cytosolic $[\text{Ca}^{2+}]_i$ by either extruding Ca^{2+} from the cell, i.e. sarcolemma Na^+ - Ca^{2+} exchangers and Ca^{2+} ATPase's, or refilling the SR, via the SERCA pumps. The majority of the cytosolic $[\text{Ca}^{2+}]_i$ is taken to refill the SR while the rest is extruded from the cell. The important thing is that Ca^{2+} must be kept at a steady state, meaning the Ca^{2+} entering the cytoplasm, either from the SR or extracellular space, during the contraction phase must equal the amount of Ca^{2+} leaving the cytoplasm during the relaxation phase [35], since an unbalance in the steady state of Ca^{2+} leads to congestive heart failure.

Although Ca^{2+} mishandling can occur due to many reasons, it is the decrease in the SR Ca^{2+} stores that often leads to congestive heart failure. The decrease in SR Ca^{2+} can be caused by several abnormalities in the influx and efflux Ca^{2+} mechanisms. Down regulation of SERCA and up regulation of NCX are two of the most common affect seen in heart failure [36]. The upregulation of NCX seen in heart failure is mainly attributed to increased mRNA and protein levels. On the other hand, the downregulation of SERCA is only partially credited to reduced levels of SERCA2a. The down regulated SERCA may also be caused by a decrease in the phosphorylation of phospholambin which inhibits SERCA until phosphorylation occurs [37]. Up regulation of NCX causes the majority of Ca^{2+} to exit the cell disrupting the steady state balance of Ca^{2+} within the myocytes. The down regulated SERCA further struggles to refill its stores due to the decrease in cytosolic $[\text{Ca}^{2+}]_i$ by NCX. In the typical model of heart failure, diminished cytosolic $[\text{Ca}^{2+}]_i$ causes inadequate Ca^{2+} binding to troponin C which fails to illicit a heart contraction. However, studies have demonstrated that the decrease in SERCA can be overcome by a significant increase in NCX function leading normal diastolic function [38].

Another area of research being investigated is the effect of Ca^{2+} leakage through the ryanodine receptors on the SR. The ryanodine receptor is often hyperphosphorylated at Ser-2809 by PKA in heart failure, which is mainly due to a decrease in phosphatases and phosphodiesterases activity which remove phosphates groups from proteins [39]. Phosphorylation of the ryanodine receptor causes dissociation of the protein calstabin allowing ryanodine to leak Ca^{2+} from its internal SR stores. In addition, ryanodine receptors

can also be phosphorylated by CAMKII at Ser 2815 which allows calstabin to dissociate and initiate Ca^{2+} leakage [40]. These two areas of research are being investigated as possible drug therapies for heart failure. The use of small-molecule inhibitors of CAMKII and stabilizers of calstabin may aid in the regulation ryanodine receptors by maintaining the complex that holds ryanodine in the closed conformation, thus inhibiting Ca^{2+} leakage [41].

Conclusion

Ca^{2+} is one of the most diverse and well researched second messenger molecules found in the cell. Ca^{2+} function varies greatly depending on cell type and local Ca^{2+} concentrations, which can initiate many physiological functions, such as muscle contractions to apoptosis. Similarly, several mechanisms are present that can regulate Ca^{2+} homeostasis. Disruptions in Ca^{2+} handling can contribute to the pathogenesis of many diseases such as Alzheimer's disease, Huntington's disease, and congestive heart failure. Thus, identification of the unique Ca^{2+} channels and research on proteins that are involved in Ca^{2+} signaling and its deficiencies in Ca^{2+} handling may lead to the development of drug therapy to combat the symptoms or possibly the disease itself.

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Fig. 6.1.

Ca²⁺ signaling: This cartoon illustrates the intricate balance between several channels and proteins that regulate Ca²⁺ signaling. Activation of Ca²⁺ signaling initiates either via agonist binding to the receptors (GPCR/RTK) that results in the generation of cellular messenger IP₃ or by alterations in the membrane potential that activates the voltage-gated Ca²⁺ channels (VGCC). Binding of the ligand to the AMPA and NMDA receptors also directly activate these channels that bring Ca²⁺. IP₃ binding to its receptor (IP₃R) in the ER depletes ER Ca²⁺ stores and activates store/receptor operated Ca²⁺ channels (SOCC/ROCC). Activation of these Ca²⁺ channels raises the [Ca²⁺]_{cyt} which not only aid in the SERCA pump-mediated ER store refilling but also promotes the regulation of several cellular functions as highlighted in the figure. Additionally, other transporters (such as NCX, NCKX) along with the activity of PMCA further remove Ca²⁺ from the cytosol and assist in maintaining low [Ca²⁺]_{cyt} levels

Fig. 6.2.

Characterization of Ca^{2+} channels: IP_3 - and EGTA-induced currents in macrophage cells. 10 μM IP_3 and 10 mM EGTA were introduced through patch pipette into the cells. (a), indicated the inward currents measured at -80 mV and (b), indicates the I-V curve exhibiting inward rectification. Tg induced currents in SH-SY5Y cells are shown in (c) and the I-V curve is close to linear which is different from macrophage cells (d). A representative current trace from primary hippocampal neurons, elicited by 150 ms test pulses in 10 mV increments from a holding potential of -70 mV to potentials between -50 and $+50$ mV is shown in (e). I_{Ca} -voltage relationship of the voltage-gated channels is shown in (f). Current amplitudes were normalized to cell capacitance and plotted as mean values