Chapter 31
Emerging Roles of Canonical TRP Channels in Neuronal Function

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Abstract Ca^{2+} signaling in neurons is intimately associated with the regulation of vital physiological processes including growth, survival and differentiation. In neurons, Ca^{2+} elicits two major functions. First as a charge carrier, Ca^{2+} reveals an indispensable role in information relay via membrane depolarization, exocytosis, and the release of neurotransmitters. Second on a global basis, Ca^{2+} acts as a ubiquitous intracellular messenger to modulate neuronal function. Thus, to mediate Ca^{2+}-dependent physiological events, neurons engage multiple mode of Ca^{2+} entry through a variety of Ca^{2+} permeable plasma membrane channels. Here we discuss a subset of specialized Ca^{2+}-permeable non-selective TRPC channels and summarize their physiological and pathological role in the context of excitable cells. TRPC channels are predominately expressed in neuronal cells and are activated through complex mechanisms, including second messengers and store depletion. A growing body of evidence suggests a prime contribution of TRPC channels in regulating fundamental neuronal functions. TRPC channels have been shown to be associated with neuronal development, proliferation and differentiation. In addition, TRPC channels have also been suggested to have a potential role in regulating neurosecretion, long term potentiation, and synaptic plasticity. During the past years, numerous seminal discoveries relating TRPC channels to neurons have constantly emphasized on the significant contribution of this group of ion channels in regulating neuronal function. Here we review the major groundbreaking work that has uniquely placed TRPC channels in a pivotal position for governing neuronal Ca^{2+} signaling and associated physiological responses.

31.1 Introduction

Both release of Ca^{2+} from intracellular stores as well as Ca^{2+} influx across the plasma membrane (PM) plays an important role in regulating cellular processes that range from cell division to cell death [1]. In neurons, Ca^{2+} plays a seminal

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role as a charge carrier and is an essential intracellular messenger, which could link brain function to cellular changes in humans and other multicellular organisms. Stimulation of neuronal cells using various agonists or pharmacological agents lead to an increase in intracellular Ca^{2+} ($[Ca^{2+}]_i$) [2,3]. This increase in $[Ca^{2+}]_i$ that is attributed from both release of Ca^{2+} from intracellular ER stores as well as Ca^{2+} entry across the membrane via the TRPC channels (Fig. 31.1 outlines the activation mechanism of TRPC channels). Although in most of these processes release of intracellular Ca^{2+} stores is critical, it is the influx of external Ca^{2+}, which is always essential to have a global or sustained response. Furthermore, Ca^{2+} influx followed by ER store-depletion accomplishes several critical cellular functions. First, this Ca^{2+} influx replenishes the ER Ca^{2+} stores, thereby, maintaining its ability to release Ca^{2+} upon subsequent stimuli. Second, since ER has limited Ca^{2+} capacity, Ca^{2+} influx is essential for increasing $[Ca^{2+}]_i$ levels to have a physiological response. Third, since Ca^{2+} concentrations within the ER must be maintained at sufficient levels in order for the organelle to carry out many of its fundamental functions, it could be anticipated that chronic depletion of ER Ca^{2+}, as would occur in the absence of Ca^{2+} influx via the TRPC channels, could not only influence ER-dependent processes such as protein folding and trafficking, but could also inhibit cellular functions that are dependent on increase in $[Ca^{2+}]_i$.

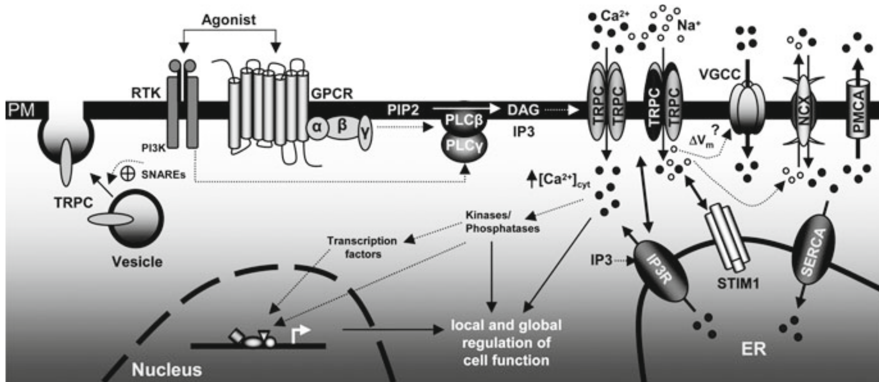

Fig. 31.1 General mechanism of TRPC channel activation: Agonist-mediated stimulation of RTKs or GPCRs initiate a signaling cascade leading to PLC-mediated hydrolysis of membrane bound PIP2, generating IP3 and DAG. DAG remains membrane associated, and could directly activate certain TRPC channels, whereas IP3 diffuses across the cytoplasm to activate its cognate-IP3 receptor. Activation of IP3R initiates ER Ca^{2+} store depletion. The Ca^{2+} sensor STIM1 senses the store-depletion and subsequently activates TRPC channels. IP3R also tends to interact with and activate homo/heteromeric TRPC channels. Additionally, the activation-dependant PM insertion of TRPC channels constitute a unique parameter for channel activation. PI3K and SNARE proteins are shown to positively regulate this trafficking. Thus, the resulting elevation in cytoplasmic $[Ca^{2+}]_{cyt}$, ensuing activation of TRPC channels, orchestrate cellular functions. It is also depicted that TRPC-mediated non-selective ionic conductance could depolarize membrane and presumably activate voltage-gated Ca^{2+} channels (VGCC) as well as NCX. PMCA and SERCA pumps are shown to work concertedly to maintain steady-state levels of intracellular Ca^{2+}

Ca^{2+} levels have been shown to be critical for gene regulation, muscle contraction, neurosecretion, integration of electrical signaling, neuronal excitability, synaptic plasticity, neuronal proliferation, and apoptosis-mediated neuronal loss. Although several mechanisms are known to control Ca^{2+} influx across the plasma membrane, Ca^{2+} influx could be more directly controlled either by store-depletion *per se* or by the alterations in the membrane potential which activates the voltage-gated Ca^{2+} channels. Since, Ca^{2+} regulates such diverse processes, it could not be attributed to one particular Ca^{2+} channel and factors such as amplitude, amount of cytosolic Ca^{2+}, spatial distribution of individual Ca^{2+} channels and regulators, may indeed be critical for regulating these diverse processes [2]. Furthermore, a set point for Ca^{2+} is perhaps critical to maintain normal physiological response and alterations in this Ca^{2+} set point could tilt the balance, thereby resulting in certain pathological conditions such as Alzheimer disease (AD) and Parkinson disease (PD). Although the significance of voltage-gated Ca^{2+} channels in neuronal cells is quite apparent, evidence suggesting an equally important role of the Transient receptor potential canonical (TRPC) channels is gaining momentum. Thus, the extraordinary ability of TRPC channels in regulating neuro-physiology is being discussed in the following sections.

31.2 Physiological Importance of Canonical TRP Channels in Neurons

In mammalian system, TRPC channels constitute a sub-group of the family of ion channels that comprises of 28 members (divided into TRPC (Canonical/Classical), TRPV (Vanilloid), and TRPM (Melastatin) sub-families) that are conserved and share significant homology among them [4]. A unique property of these channels is that they function as non-selective Ca^{2+} entry channels, with distinct mode of activation [4]. TRPC family contains 7 members (C1-C7) which, based on their similarities in structure-function relationships, are further divided into two sub-groups. The first group consists of C1/C4/C5 channels that are activated primarily by store-depletion. The second group comprises of C3/C6/C7 that are activated by receptor stimulation [5]. C2 is a pseudogene in humans, but has immense role in rodent behavior and pheromone sensing [6]. It is important to note that, although one can pharmacologically separate these channels in vitro, their activation in a physiological context is linked to PLC mediated signaling following stimulation of membrane G-protein coupled receptors (GPCRs) or receptor tyrosine kinases (RTKs) (Fig. 31.1). Further, since TRPC proteins are capable of forming functional channels by heteromeric interactions, the receptor- or store-dependant activation thus outlines a common feature in channel activation.

Ca^{2+} entry via the G-protein coupled mechanism has been implicated in the shaping of action potentials, synaptic transmission, and sensory transduction

[7, 8]. Additionally, changes in $[Ca^{2+}]_i$ is known to regulate the motility of many cellular structures, including the axonal growth cones [9] and dendritic filopodia of developing neurons [10]. Thus, it can be anticipated that TRPC channels may have a significant role in regulating these fundamental neuronal processes. Indeed, Ca^{2+} entry through TRPC channel has been shown to play a critical role in basic fibroblast growth factor (bFGF) induced cortical neural stem cells (NSCs) proliferation [11]. It has been shown that activation of C1, but not C3 regulate cell proliferation in neural stem cells. In contrast, Ca^{2+} influx through C1 and C3 controlled the switch between proliferation and differentiation in immortalized hippocampal H19-7 cells [12]. Similarly in mammals, C3 and C6, but not C1, have been shown to be associated with BDNF-mediated neuronal growth [13]. These distinct physiological outcomes can be explained by the differences in spatial localization and functional activation of individual TRPC channels in diverse neuronal population. Additionally, the differential distribution of membrane receptors (GPCRs/RTKs), concentration and availability of the desired agonist can further add to the complex regulation of distinct set of TRPC channels.

31.2.1 TRPC1

TRPC1 (C1), the founding member of canonical TRPs, has been shown to be expressed in both neuronal and non-neuronal tissues [14]. The first report to identify the role of C1 channels in neuronal function came from David Clapham's group. This group indicated that C1 and C5 were expressed in the hippocampus and their co-expression resulted in a novel non-selective cation channel with voltage dependence similar to NMDA receptor channels [15]. Furthermore, C1 expression was only observed in neuronal cells, with no functional activity and no localization of C1 was observed at the synapse [15]. However, another report from Paul Worley's group suggested that C1 was expressed in perisynaptic regions of the synapse and was physically associated with mGluR1 [16]. Furthermore, expression of a dominant-negative C1 – pore mutant (F561A) in cerebellar Purkinje neurons, resulted in a 49% reduction of mGluR-evoked slow excitatory postsynaptic currents (EPSCs) whereas fast transmission mediated by AMPA-type glutamate receptors remained unaffected, indicating that mGluR1 receptor activation is essential for the gating of C1. Similarly, in dopaminergic neurons C1 was functional and important for Ca^{2+} entry upon store-depletion [17]. The only explanation for these opposite results could be that in different neurons, C1 expression and function might be regulated differentially. It is possible that in hippochampal neurons C1 may not be routed to the membrane (synapse), whereas in purkinje or dopaminergic neurons it could. Also since Clapham's group did not find a functional C1 channel activity, whereas other groups were able to measure C1 function in different neurons [17, 18], suggesting that perhaps routing of C1 channels was altered in certain neuronal population. Interestingly, recently it has been shown that Caveolin1 is critical for PM routing of C1 channels [19, 20] indicating that C1 might interact with

other proteins that could be critical for its localization and function. Another report indicated that C1 channels were primarily expressed on cell soma and on dendrites, whereas C5 channels were exclusively located on cell bodies [21], which support the idea that different neuronal population would have different mechanism for the expression as well as for the routing of these TRPC channels. Besides brain, C1 has also been shown to be expressed in retina [22, 23]; however its function in these tissues is not yet defined. Importantly, C1 was also found to be localized at the peripheral axons and the mechanosensory terminals, thus the involvement of C1 channels in mechanotransduction can be anticipated [24]. However, future research is needed to confirm these key findings.

Ca^{2+} entry is essential for cell proliferation and differentiation and C1 has been shown to be critical for proliferation of various non-excitable cells [5, 25], however its role in neuronal proliferation warrants elaborate investigation. Wu et al., showed for the first time that in hippochampal H19-7 cell line, C1 and C3 were essential for switching from proliferating to differentiating phenotype. Importantly, C1 was highly expressed in embryonic CNS in mammals than adult, indicating that C1 could be involved in early development and proliferation of neurons [12]. Furthermore, blocking of TRPC channels or silencing of C1 alone attenuated bFGF induced intracellular Ca^{2+} elevation and NSC's proliferation [11]. In contrast, it has been also shown that C1 is required for midline guidance of axons of commissural interneurons, and angiogenic sprouting of intersegmental vessels in the developing *Xenopus* spinal cord, but not in regeneration of adult Xenopus spinal cord, suggesting that C1 may serve as a key mediator for the Ca^{2+} influx that regulate axonal guidance during development, but inhibit axonal regeneration in adulthood [26]. Importantly, C1 homologue has also been shown to mediate BDNF-activated Ca^{2+} increase and regulate growth cone turning and extension in *Xenopus* spinal neurons [27]. Although there are slight discrepancies with regard to proliferation vs regeneration, the differences could be attributed to different neuronal populations that could have different expression of TRPC isoforms, which could form heteromultimers, and could therefore function differently in different tissues. Furthermore, stimulation of C1 via different agonists could very well have a different physiological response that could be attributed with spatial temporal resolution of Ca^{2+} signaling. Interestingly, C1, C2 and C4 had been shown to exhibit higher expression, whereas C3 and C6 expressions were decreased in NSC population [11]. These results suggest that perhaps in NSC cells, C1 could associate with C4 rather than C3 or C5, as observed above, and thus could bring about a different physiological function. Interestingly, in most of the cases the survival and proliferation induced by C1 appears to be dependent on its Ca^{2+} entry; however it remains to be seen if C1 could potentially regulate other proteins, independent of its Ca^{2+} influx ability. This could be true, since recently it was shown that in muscle cells C1 was localized in the ER/SR compartments [28] and also C1 has been shown to form large protein-protein complexes [19, 29]. Although C1 has been implicated in neuronal function, its role using animal models is not yet established. C1 knockout mice are born healthy and survive in control environment and it would be interesting to confirm the function of C1 channels in neuronal functions.

31.2.2 TRPC2

TRPC2 (C2) is a unique member of TRPC sub-family, since its expression in mammals is lost and is considered as a pseudogene in higher mammals; however C2 has been shown to be essential for small rodents and oocytes. In rodents, C2 is exclusively expressed in the vomeronasal sensory system (VNO neurons) and is localized to the dendritic tip of VNO, which are important for pheromone sensory transduction [30]. Importantly, the mouse VNO has been shown to mediate social behaviors and male mice that are deficient in C2 expression failed to display male-male aggression, and showed sexual and courtship behaviors toward both males and females [30]. C2-deficient mice also showed decreased action potential recordings of sensory responses induced by pheromonal cues in the VNO. Furthermore, another study showed that C2 knockout males increased their efforts to mount other males and demonstrated inappropriate behavior [31]. These results suggest that in rodents sensory activation of the VNO is essential for sex discrimination that ensures gender-specific behavior. Although VNO is known to be important for sensation, it could not be the only reason why these knockout mice showed behavioral issues. Importantly, it has also been shown that C2 knockout female mice had higher testosterone levels, which could be responsible for some of these behavioral issues.

C2 has also been shown to be critical for the activation of the acrosome reaction in oocyte's [32]; however since C2 knockout mice were able to reproduce normally and have similar number of offspring's, it is unlikely that C2 is the only channel that could provide Ca^{2+} needed for acrosome reaction in rodents. In contrast, C2 could still be critical in the fertilization of oocytes and more research is needed to identify if other TRPC could possibly compensate for C2 function in the fertilization of rodents. In addition, recent report also indicated that arachidonic acid generated by PLC activation in the VNO neurons activate two types of channels, a C2 channel and a separate Ca^{2+}-permeable channel [33]. Although the identification of this second channel is not known, it could be anticipated that other TRPC (mainly C3 or C6) that are expressed in these neurons could contribute to these currents. Although humans do not express C2 channels and do not have similar VNO neurons (are degenerated), but they posses a functional olfactory system and it could be suggested that perhaps another TRPC isoforms could decode olfactory cues and could be responsible for normal and abnormal social behavior in humans [34]. Thus, it would be interesting to probe this research further and establish if other TRPC isoforms are involved in abnormal human behaviors.

31.2.3 TRPC3

TRPC3 (C3) is expressed in both non-excitable and excitable cells and displays remarkable potential to form homo and hetero oligomers with different members of the TRPC family [35]. Though the expression of C3 is highest in the brain the functional role of C3 in the nervous system is not completely understood. Pioneering work on C3 in neurons revealed a role for C3 channels in BDNF signaling [36].

In pontine neurons it was shown that Trk receptors and C3 are expressed during the same developmental stages of the brain and BDNF-stimulated non-selective cationic current (I_{BDNF}) was mediated by C3. Similarly, in cerebellar granule neurons (CGNs), BDNF-induced elevation of Ca^{2+} contributes to axonal growth cone guidance [37]. The specific involvement of C3 in BDNF-induced growth cone plasticity was further demonstrated by silencing endogenous C3 or by expressing a dominant-negative C3. Furthermore, in the pyramidal neurons of rat hippocampus an involvement of C3 channels in BDNF-induced dendritic spine formation has also been reported [38]. Importantly, I_{BDNF} was blocked by antibodies directed against C3 or by C3 knockdown, which decreased BDNF, induced dendritic spine density, suggesting that C3 function as mediators of BDNF-initiated dendritic remodeling. Apart from the rapid local effects of growth cone guidance and dendritic spine morphogenesis, BDNF also has a prime role in neuronal survival. In CGNs, C3 is shown to have a protective role against serum-deprivation induced cell death, as overexpression of exogenous C3 prevented apoptosis and down-regulation of C3 induced apoptosis. This was further shown to be mediated by BDNF-induced CREB activation since the protective effect of C3 was blocked by the expression of a dominant-negative form of CREB [13]. Further, in H19-7 rat hippocampal neuronal cells C3 along with C1 was significantly increased under differentiating condition, whereas the expression of C4 and C7 were decreased [12]. Thus, this reciprocal regulation of TRPC channel expression in these neurons strongly suggests their developmentally important function.

In an interesting study performed to understand the role of Calcinuerin (CaN)-NFAT signaling pathway in neuromuscular activity, it was found that C3 channel expression was enhanced in response to neurostimulation and CaN activation. Both neurostimulation and CaN activate NFAT, but to maintain the translocation of NFAT to nucleus and modulate the gene expression a sustained Ca^{2+} influx is required. Thus, a feed back loop is established wherein the activated NFAT further enhances the expression of C3 and helps maintain the sustained flow of Ca^{2+} [39]. Additionally, recent reports from two independent studies have demonstrated a physiologically significant role of C3 channels in motor coordination [40, 41]. In one of the studies using mouse cerebellar Purkinje cells, the authors established that C3 is required for the slow synaptic potentials and inward currents evoked by group I metabotropic receptor (mGluR1) synaptic signaling. In mice lacking C3, but not C1, C4 or C6, the mGluR1 mediated slow synaptic potentials were completely absent. This abnormality in the glutamate neurotransmission in the post-synaptic neurons has resulted in impaired walking behavior thus establishing a fundamental role for C3 channels in motor coordination [41]. In yet another study, to identify crucial gene products implicated in cerebral ataxia, phenotype-driven dominant mutagenesis screen was performed and an ataxic mouse mutant by the name moonwalker (Mwk) mice was identified [40]. These mice exhibited a gain-of-function mutation (T635A) and maintained sustained activation of C3 channels due to lack of negative feedback regulation by PKCγ-mediated phosphorylation. As a result diminished dendritic arborization and progressive loss of Purkinje neurons was observed. Although these studies contradict each other, they still suggest that

C3 could have a pivotal role in Purkinje neurons and future studies are needed to confirm the role of C3 in ataxia. Corroborating these results is another study with a C3 knockout mouse model, where the C3 promoter region was disrupted. These mouse exhibit atrophy and progressive paralysis suggesting an obligatory role of C3 channels in neuronal signaling, differentiation and development [42]. Importantly, C3 has been shown to associate with SNARE complex proteins [43], indicating that C3 could be important for neurosecretion.

31.2.4 TRPC4

TRPC4 (C4) is not only expressed in brain, but is also expressed in endothelium, kidney, retina, testis and adrenal glands [44]. However, the mode of activation of the channel may differ in these cell systems. C4 has shown to be co-expressed with C5 in CA1 pyramidal neurons of the hippocampus and have been shown to participate in neuronal Ca^{2+} homeostasis, however direct function of C4 was not tested in this report [45]. To elucidate the function of C4 channels, Nowycky and colleagues showed that C4 channels could provide sufficient Ca^{2+} influx to trigger a robust secretory response in voltage-clamped neurosecretory cells, indicating that C4 by itself or in combination with other TRPC channels has the potential to regulate exocytosis [46]. In addition, gamma-aminobutyric acid (GABA) release from thalamic interneurons by the activation of 5-hydroxytryptamine type 2 receptors requires Ca^{2+} entry that was critically dependent on C4, further indicating that C4 could have a role in regulating neurotransmitter release. Importantly, C4 knockout mice showed reduced GABA release from the thalamic interneurons upon 5-hydroxytryptamine stimulation, but not by acetyl-ß-methylcholine [47]. These results suggest that perhaps the F2 terminals are still functional in C4 knockout mice, and the regulation of GABA release via different mechanism may be critical for specific functions, such as the sleep–wake cycle and processing of visual information [48].

C4 expression was shown to be restricted to granule and their precursor cells in rat cerebellum, which was decreased in adults [49], suggesting that C4 is required for proper granule cell development. In contrast, in adult rat dorsal root ganglia, nerve injury mediated neurite outgrowth was dependent on C4 expression, indicating the involvement of C4 channels in axonal regeneration following nerve injury. In addition, C4 has also been shown to have a role in acute and delayed neuronal injury in focal cerebral ischemia [50]; however these studies were only based on C4 overexpression thus, further research is needed to confirm these findings. Although both these studies were performed in different neurons, they demonstrate a unique feature of C4 channels being critical for neuronal development especially during nerve injury. Although the mechanism via which C4 channels regulate neurite outgrowth is not yet identified, it could be suggested that certain kinases that are activated by increased Ca^{2+} may in turn regulate gene expression and thereby increase neurite outgrowth. Although C4 knockout mice caused impairment in store-operated Ca^{2+} currents in endothelial cells and also showed that γ-aminobutyric acid (GABA)

release from thalamic interneuron's was inhibited, studies suggesting its role in neuronal cell proliferation, preferentially using the C4 knockout mice, is still lacking. Thus future research in this direction is warranted to unequivocally establish the role of C4 channels in neuronal function.

31.2.5 TRPC5

TRPC5 (C5) has been demonstrated to functionally exist as homomultimeres or in heteromultimeric assembly with other TRPC proteins. Distinct from its receptor- or store-dependant route of activation, C5 channels can also be readily activated by lipids such as lipophosphatidylcholine and sphingosine 1-phosphate, thus indicating toward a lipid-sensing ability of the channel [51]. Interestingly, the concept of rapid vesicular translocation and PM insertion has been suggested as a mode of C5 activation. EGF stimulation of hippocampal neurons is shown to target vesicle-associated C5 channels to the PM, this enables in maintaining a sustained Ca^{2+} influx and aids in neurite remodeling [52, 53]. In neuronal cell development, establishment of morphological and functional polarity is a critical feature in the formation of neural circuits and for efficient transmission of information across neurons [54]. In cultures, establishment of neuronal polarity is divided into five stages – stage 1 formation of lamellipodia, stage 2 development into neurites, stage 3 extension of one of the neurites to form axon, stage 4 dendritic arborization and stage 5 spine/synapse formation [55]. During development, every stage involved in compartmentalizing the neuron utilizes Ca^{2+} and its downstream signaling molecules to perform varied complex processes. Intracellular Ca^{2+} has diverse effects in shaping up the axons and dendrites. One of the downstream effectors of Ca^{2+} are Ca^{2+}/CaM dependent kinases (CaMK) which function by association with Ca^{2+}/CaM, an intracellular Ca^{2+} sensor. The action of CaMK in axon formation has been shown to be promoted by the Ca^{2+} influx via C5 channels, since knockdown of endogenous C5 suppresses CaMKK mediated activation of CaMKγ and thus axon formation [56]. According to a Ca^{2+} set-point hypothesis growth cone motility and neurite extensions depend on an optimal range of $[Ca^{2+}]_i$, concentrations above or below the optimal range, that alter the Ca^{2+} homeostasis, is shown to retard the growth cone protrusion [57]. On the contrary, growth cone steering induced by various extracellular guidance cues require localized Ca^{2+} influx. C5 has been shown to be a candidate protein which produces Ca^{2+} transients at resting membrane potentials [58]. In a study by Clapham's group an inverse correlation has been shown between C5 expression and hippocampal neurite length and growth cone morphology [58]. Point to note here is that C3 and XTRPC have been shown to play an opposite effect in CGNs and *Xenopus* spinal neurons respectively, wherein they both promote chemotropic turning of growth cones, suggesting different Ca^{2+} requirements for various processes.

It has been suggested that crosslinking of GM1 ganglioside associates with α5β1 integrin, which in turn initiates PLCγ and PI3K signaling cascade through auto-phosphorylation of Focal adhesion kinase (FAK) tyrosine kinase [53]. Upon

activation of this signaling cascade neurite out-growth was accelerated. Further, this study identified C5 protein on cell bodies of CGNs and demonstrated C5 channels as a critical modulator of this PLC-pathway mediated neurite outgrowth. C5 knock-out mice were shown to exhibit diminished innate fear levels in response to innately aversive stimuli. It has been reasoned that the lack of C5 channel potentiation by Group I mGluRs and/or CCK2 receptors and subsequent lack of membrane depolarization prevents the transmission of information to output neurons of the innate fear circuitry thus, resulting in the above mentioned fear-related behavior [59]. C5 channels were shown to have a role in generating Ca^{2+}-activated slow afterdepolarization (sADP) currents signaled by muscarinic receptors [60]. Overexpression of C5 facilitates I_{sADP} whereas expression of a pore-dead C5 dominant-negative mutant inhibits $I_{sADP.}$ The authors argue that the afterdepolarization current generated by TRPC channels is not a result of the mere Ca^{2+} influx through the channels, but more likely due to the membrane depolarization, mediated by Na^{+} entry, capable of activating voltage-dependent Ca^{2+} channels. These afterdepolarization currents allow the cell to maintain the transient input signals for a sustained period leading to long-lasting changes in excitability.

31.2.6 TRPC6

In mammals, TRPC6 (C6) is widely expressed in the cardiac neurons [61], retinal ganglion cells [62], in the neurons of olfactory epithelium [63] and in parts of brain such as – cortex, substantia niagra, hippocampus and cerebellum [49, 64–66]. Both in excitable and non-excitable cells, C6 channels largely constitute the receptor-operated, non-selective cation channels. It has been shown that receptor stimulation of PLC pathway or direct application of DAG and its analogues could specifically initiate Ca^{2+} entry [67, 68]. Importantly, this store-independent activation of C6 channels has been specifically used as readout for PLCβ activation in endocannabinoid signaling [69]. Interestingly, C6 channels could also be selectively activated by – hyperphorin [70, 71], fulfenamic acid [72] and tyrosine phosphorylation [73, 74]. Activation of Neurokinin receptors by substance-P in the noradrenergic A7 neurons has been shown to bring about a C6-specific non-selective cationic conductance, thus providing evidence for the involvement of C6 channels in nociception [75]. Regulation of TRPC channel-function has been attributed to the channel assembly into macromolecular complexes [19, 29]. In PC12 cells, activation of muscarinic GPCR has been demonstrated to organize a C6 centered channel-complex, depicting C6 in association with a multi-protein complex containing M1AChR, Calmodulin (CaM), immunophilin FKBP12, Calcinurin (CaN) and PKC [76]. Interestingly, this study also demonstrates TRPC6 to be phosphorylated by PKC and de-phosphorylated by CaN, thus regulating the channel activity. Similarly, in PC12 cells and rat cerebral cortex, a molecular complex of C6 with the GPCR – α_{1A}-adrenoceptor (α_{1A}-AR) and Snapin (a synaptic vesicle associated protein) has been reported, where following the activation of α_{1A}-AR, increased

association of C6 with Snapin and α_{1A}-AR facilitates the PM recruitment of C6, thereby resulting in C6-mediated Ca^{2+} entry [77]. Additionally, in rodent rod cells, such C6 channel-complex has been shown to compartmentalize Ca^{2+} influx process distinct from that of voltage gated Ca^{2+} channels [78].

C6 channels have also been functionally associated with aggressive phenotype and growth of glioblastomas [79]. In cells derived from glioblastomas, hypoxic stress leads to Notch1-mediated upregulation of C6, which amplifies a CaN/NFAT pathway critical for cancerous growth. Further, NGF stimulation of PC12 cells results in the overexpression of C6 and is shown to be involved in the non-transferin mediated iron uptake [80]. A similar upregulation of C6-mediated Ca^{2+} influx has been reported in cultured mouse cortical astrocytes following chronic IL-1β treatment [81]. The role of C6 channels in neuronal function seems to be contextual, as these findings which indicate toward a potential involvement of C6 in neuronal toxicity and inflammation mediated neurodegeneration, there are several other significant findings that have enumerated an indispensable function of C6 in maintenance of normal neuronal physiology. C6 has been shown to co-operate with other TRP channels, in a heteromeric assembly, to regulate critical neuronal functions. The significance of heteromeric C6 channels in brain development is underscored by the finding that, in embryonic rat brain C6 physically associates with C1, C4 and C5 respectively [82]. In dorsal root ganglion (DRG) neurons, C6 co-operates with TRPC1 and TRPV4 to regulate nociception [83]. C6, in conjunction with C3, is involved in growth-cone guidance [37]. In rat cerebellar granule neurons, BDNF-induced activation of TrkB receptors resulted in a Ca^{2+} dependant growth-cone turning. BDNF-induced Ca^{2+} influx was shown to be mediated via C3/C6 channels. Since, silencing of C3, but not C1, and expression of dominant-negative constructs of C3 or C6 respectively inhibited neuronal attraction toward a BDNF gradient. Similarly, C6 together with C3 [13] and C5 [84] have been reported to collaborate in neuronal survival. In CGN, both C3 and C6, but not C1, were shown to elicit the neuroprotective effect of BDNF via the activation of CREB/ERK pathway [13]. In primary mid-brain neurons of rat, C5 and C6 co-localized with PDGF-βR and were found to regulate PDGF-mediated neuronal cell survival following HIV-1 Tat toxicity [84]. In addition to its pro-survival characteristic, C6 channels have been demonstrated to regulate dendrite development, thus influencing synaptic plasticity. In rat hippocampus the expression of C6 was detected postnatal, which peaked between postnatal day 7 through day 28 (P7-28). C6 was enriched in the synaptsomes and postsynaptic cell fractions obtained from P14 hippocampus and also significantly co-localized with PSD-95. Using immuno-electron microscopy, this study identified a predominant localization of C6 at the excitatory synapse of the hippocampal neurons. Increased expression of C6 enhanced the dendritic spine densities, whereas specific C6 silencing, but not C1, significantly reduced the dendritic spine densities. C6 silencing also abolished the effect of BDNF on spine formation. This effect of C6 channels on dendritic spine formation was largely mediated by the activation of the CaMKIV-CREB pathway. In agreement to the function of C6 in culture hippocampal neurons, overexpression of C6 in vivo also resulted in an increase

in the dendritic spin densities in CA1 neurons, further emphasizing a significant function of C6 channels in the formation of excitatory synapse in rat hippocampal neurons. In addition, the C6 transgenic animals displayed an enhanced ability for learning and memory [85, 86].

31.2.7 TRPC7

TRPC7 (C7) was cloned from mouse as well as human brain [87, 88] and is thus far the last member of canonical TRPCs to be identified. Among other TRPC homologues, C7 displays the closest structural resemblance with C3 and together with C6 they constitute the receptor-activated subfamily of TRPC channels [88, 89]. In humans the highest level of C7 message is detected in the pituitary gland [66, 88]. In mouse brain, C7 is prominently expressed in the cerebellar purkinje cells in addition to hippocampal neurons, cerebral cortex, pons and the mitral layer of olfactory bulb [87]. C7 expression has also been detected in rat hippocampal neurons [12]. In spite of such a broad expression pattern of C7 in the CNS, the neuronal functions of the channel remain elusive. The existing literature, however reveal some critical observations that associate C7 channels with neuronal physiology. C7 is expressed in the rat striatal cholinergic interneurons and along with C3 has been demonstrated to develop TRPC-like currents, in response to activation of the Gαq-coupled mGluR1/5 [90], thus implying towards an involvement of C7 ion channels in regulating striatal function. In the proliferating rat H19-7 hippocampal neurons the expression of C7 was high and following differentiation the expression was dramatically reduced [12]. Although in this study C7 did not essentially contribute to SOCE, the existence of such a switch in the expression pattern of C7 and other TRPC proteins does indicate towards an internal mechanism of neurons to control physiological processes by regulating TRPC channel function. One of the cloning studies mapped the C7 gene to human chromosome 21q22.3 and surprisingly this locus is associated with bipolar disorder, thus indicating a possible involvement of C7 in the pathophysiology of bipolar disorder [91]. Interestingly, a reduced level of C7 has been reported in bipolar disorder patients. In the B-lymphoblast cells obtained from one group of bipolar patients, the expression of C7 mRNA was reduced, whereas the basal Ca^{2+} levels in these cells were high [92]. The authors speculate that this inverse relation between C7 expression and Ca^{2+} levels is probably an inbuilt cellular mechanism to restrict further Ca^{2+} influx through C7 channels. In addition, C7 expression has been reported in melanopsin-expressing retinal ganglion cells (RGCs) and inhibition of TRPC channels by non-specific inhibitors (2-APB and SKF96365) was shown to abolish light-evoked response of the photosensitive RGCs, thus emphasizing on a potential involvement of C7 channels in mammalian phototransduction [93]. In summary, reports demonstrating the role of C7 in neurons are limited however, since in native systems C7 has been shown to be equally important for receptor- and store-operated Ca^{2+} entry, it is apparent that in a neuronal context C7 would have an essential physiological role by co-operating with other TRPC channels in a heterotypic manner.

31.3 TRPC Proteins in Neurodegenerative Diseases

Neuronal cell injury is mediated via both increase and decrease of $[Ca^{2+}]_i$ concentration [94]. Changes in $[Ca^{2+}]_i$ concentration stimulate a number of intracellular events and could either trigger or inhibit cell death process [2, 3]. Importantly, disturbances in Ca^{2+} homeostasis have been implicated in many neurodegenerative diseases such as, PD, AD, and HD [95–99]. It is not surprising that disturbances in Ca^{2+} signaling pathways underlie neuronal loss, since many factors involved in neuronal function are dependent on Ca^{2+} signaling [2, 3]. However, the cellular mechanism(s) underlying neurodegeneration, due to alterations in Ca^{2+} homeostasis, remains to be elucidated [100]. Although several factors including generation of free radicals, impairment of mitochondrial function, ER stress, and apoptosis have been proposed, the role of TRPC channels is not directly tested.

Increased $[Ca^{2+}]_i$ could lead to inappropriate activation of Ca^{2+}-dependent processes, that stay inactive or operate at low Ca^{2+} levels, causing metabolic derangements leading to neuronal death [2, 3, 100]. Whereas, decrease of Ca^{2+} in the ER induces stress, which could activate cell death cascades [101], suggesting that controlled Ca^{2+} influx is critical for neuronal function and survival. Since, TRPCs are essential for replenishing and for maintaining ER Ca^{2+}, chronic depletion of ER Ca^{2+} as would occur in the absence of TRPC function, could influence ER-dependent processes such as protein folding and trafficking, the ER stress response, and apoptosis. This is most notable in neurons that respond to activation of the phosphoinositol pathway with $[Ca^{2+}]_i$ oscillations, since ablation of Ca^{2+} entry preclude the cell's ability to maintain $[Ca^{2+}]_i$ oscillations. Recently, it has been shown that TRPC1 levels were decreased in dopaminergic neurons when treated with neurotoxins such as 1-methyl, 4-phenyl pyridinium ion (MPP^+/MPTP) that cause parkinsonian syndrome [95, 102]. Importantly, loss of C1 decreased $[Ca^{2+}]_i$ levels and showed ER stress. In contrast, overexpression of C1 significantly decreased MPTP/MPP^+ mediated neuronal loss and also restored $[Ca^{2+}]_i$ levels, indicating that C1 could be critical for PD. Moreover, other TRPC channels are also expressed in substantia nigra, which could be involved in excitotoxicity [103]. Although these results are exciting, they need to be verified using PD mouse models and C1 knockout mice.

BDNF treatment is known to activate C3-mediated cationic current in pontine neurons [36]. Several studies have also reported that BDNF protect neurons from several brain insults thereby play an important role in neurodegenerative diseases such as AD, PD and HD [13, 100, 103], however the role of C3 is not yet directly established. Recent report also suggest that BDNF levels are negatively regulated with regard to the severity of AD and treatment with BDNF exerts neuroprotective effect in AD in vitro and in vivo models [104]; however the mechanism as well as the importance of C3 is not known. Interestingly, BDNF and NGF are known to modulate neurotransmitter release [105] and since BDNF activates C3 channels, a potential role of C3 in neurosecretion can be speculated. In contrast, Aβ has been shown to induce oxidative stress [100] and since C3 is important for oxidative stress (discussed below), it can potentially exacerbate AD. Thus, C3 could plausibly

have both protective as well as degenerative role in the context specific manner. Moreover, Lessard et al., reported that presenilins mutations account for up to 40% of the early onset of familial AD [106] and deletion of PS1 alone or PS1 and PS2 together resulted in enhanced store-dependent Ca^{2+} entry [107]. Although it is not known if TRPCs are the major culprit in familial AD, it is still intriguing that TRPC channels could have a role in the onset/progression of AD. Overall, it seems as if SOCE is the "magical pathway" since it could not only help neuronal survival, but could also induce neuronal degeneration.

31.4 TRPC Channels and Oxidative Stress

Reactive oxygen species (ROS) over production is a widespread feature for neurodegenerative disorders including PD, AD and HD [99, 108–110]. For the past several decades, a large number of studies have been elucidated the role of Ca^{2+} homeostasis in ROS production that leads to cell death [111–114]. In contrast, Amoroso et al., reported that oxidative stress induced free radical overproduction induces cell death, but without the participation of intracellular Ca^{2+} in SH-SY5Y cells [115]. The only explanation to these discrepancies could be that Ca^{2+} induced oxidative stress is cell specific and perhaps other Ca^{2+} pumps and Ca^{2+} releasing channels and intracellular buffering system might influence ROS production [116]. So far two TRPC channels (TRPC3 and TRPC4) have been identified, which are activated under oxidative stress [117, 118]. A dominant negative TRPC3 N-terminal negatively affects the TRPC3-mediated cation current and membrane depolarization induced by the oxidants tert-butylhydroperoxide (t-BHP) in porcine aortic endothelial cells (PAEC) [119]. Also during oxidative stress the C3 and C4 forms redox-sensitive cation channel and involved in Na^{+} loading and membrane depolarization in HEK293T cells [120, 121]. The mechanisms how these TRPC channels are activated by oxidative stress are not clear yet. But phospholipase c (PLC) inhibitor and tyrosine kinase inhibitor have shown to reverse the function of C3 under oxidative stress [122]. In contrast, studies performed by Crouzin et al., showed that α-tocopherol protects hippocampal neurons against Fe^{2+}-induced oxidative stress by preventing Ca^{2+} influx through TRPC-like channels [123], however the channel involved is still not yet identified and future research is needed.

31.5 Concluding Remarks

There has been intense focus on TRPC channels in the past few years, however, conclusive data regarding the exact physiological function of most of these channels in neuronal cells are still lacking. There are considerable discrepancies regarding their function, which could be due to variable expression patterns in different tissues and cells as well as their individual associations among different TRPC members. In addition their association with other accessory proteins could also be critical for their specific localization and function, which could confound the results obtained in

different neuronal tissues. Overall, although the available data reveal key functional role of TRPC channels in neurons, direct evidence from individual channel is still lacking. Although individual knockout mice are available and are healthy, the issue of compensation is never resolved or investigated. Furthermore, since TRPC family members have similar activation mechanism, it could be anticipated that TRPC homologues belonging to the same sub family could compensate for the function. Nonetheless functional experiments using multiple knockout mice will be essential to decipher the role of TRPC channels in neuronal cells. Identifying specific endogenous agents that can selectively activate a given TRPC channel remains to be an outstanding challenge.

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