Physiological Roles of the TRPM4 Channel Extracted from Background Currents

Calcium-activated nonselective cationic currents have been known for 30 years, but their physiological implications have remained unresolved until the recent cloning of the TRPM4 ion channel. Since then, TRPM4 has been identified as a key modulator of numerous calcium-dependent mechanisms such as the immune response, insulin secretion, cerebral artery constriction, respiratory rhythm, and cardiac conduction.

At the beginning of single-channel recordings, a Ca$^{2+}$-activated nonselective cationic (NSC$_{ca}$) current was described in cardiac tissue (12). Until the improvement of the patch-clamp technique by Hamill et al. (30), such currents were barely detectable, since their nonselective permeability led them to founder in the quicksands of composite background currents. The discovery of this unitary current in cardiomyocytes initiated a long chapter devoted to the description of similar currents in other tissues. Their common characteristics are their activation by intracellular calcium and their lack of discrimination between Na$^+$ and K$^+$. Ca$^{2+}$ sensitivity was a determining step in the discovery of these currents, because rising Ca$^{2+}$ concentrations on the internal side of the membrane allow unitary currents to be unmasked.

Despite the extensive description of NSC$_{ca}$ currents in a wide variety of tissues in the 1980s to 1990s, their physiological implications remained elusive. Moreover, although the molecular identities of many other currents were uncovered during this period, no candidates were known for most of the nonselective cationic currents, including the NSC$_{ca}$. The cloning of the transient receptor potential (TRP) channel family finally led to the identification of the molecular counterparts of many of these currents. Among these, the cloning of TRPM4 by Launay et al. (36) revealed the identity of the most widespread NSC$_{ca}$. Providing the molecular tools, the cloning of this molecule led to an improved understanding of the physiological implications of this NSC$_{ca}$ channel.

It is now clearly established that TRPM4 is implicated in the immune response, insulin secretion, myogenic tone of the cerebral artery, capillary fragmentation, and respiratory rhythm regulation. Moreover, a recent report describes the first human pathology associated with an inherited TRPM4 mutation: a heart disorder (34).

In this review, we present data regarding the cellular mechanisms by which TRPM4 is able to modulate physiological processes. We focus on tissues in which the implication of TRPM4 protein has been clearly demonstrated. Tissues in which NSC$_{ca}$ currents are present but the involvement of TRPM4 has not been demonstrated will be ignored. Although we will briefly mention these two aspects, we will focus neither on NSC$_{ca}$ distribution nor on the biophysical properties of TRPM4 since these have already been extensively reviewed elsewhere (80, 85).

**NSC$_{ca}$ Currents**

NSC$_{ca}$ currents have been recorded in a large variety of tissues (80). These currents are detected in both excitable and nonexcitable cells. Indeed, NSC$_{ca}$ currents have been reported in firing cells such as Helix neurons (59, 75), mouse inspiratory neurons (46), and pacemaker cells from the mouse sinus node (16). They are observed in other excitable cells such as rat cerebral arterial myocytes (19) and rat and human cardiomyocytes (25, 29).

In parallel to these studies conducted in excitable cells, NSC$_{ca}$ currents also have been detected in epithelial systems such as the endothelium (14, 23, 63, 74), intestinal tract (70), and all along the mammalian renal tubule (11, 32, 60, 81). NSC$_{ca}$ currents are present in secretory cells of both endocrine and exocrine systems such as pancreatic $\alpha$- (42) and $\beta$-cells (9) and pancreatic acinar cells (43). An NSC$_{ca}$ current is also present in astrocytes (8). Furthermore, these currents have been reported in sensory organs such as outer hair cells (83) and vomeronasal sensory neurons (37).

Most of these NSC$_{ca}$ currents have similar biophysical and regulatory properties. For example, NSC$_{ca}$ currents display single-channel conductance between 20 and 30 pS, similar permeability to Na$^+$ and K$^+$, and almost no permeability to Ca$^{2+}$. NSC$_{ca}$ currents are activated by membrane depolarization and the rise in internal calcium but inhibited by internal ATP.
TRP Channels

In the field of ion channels, the cloning of the archetypal TRP protein in *Drosophila* in the late 1980s (47) gave birth to a large family of molecular entities that provided new candidates for a variety of currents that were uncharacterized at the molecular level. Indeed, the TRP channel family includes more than 50 members divided into seven subfamilies depending on sequence homology: TRPC (Canonical), TRPV (Vanilloïd), TRPM (Melastatin), TRPP (Polycystin), TRPML (Mucolipin), TRPA (Ankyrin), and TRPN (NO-mechano-potential). Of these, 27 members are present in humans (89).

The biophysical properties of TRP family members have been described in numerous reviews (51, 66, 84). TRPs are proteins with six transmembrane domains and tetramerize to form functional channels, thus adopting the classic structure of many cationic channels such as voltage-dependent ion channels. They also share with these channels the P-loop between transmembrane segments 5 and 6, which forms a selectivity filter. TRPs are considered voltage-dependent channels; however, they possess only few positively charged amino acids in the S4 transmembrane domain that forms the sensor of the voltage-dependent ion channels.

A great majority of TRPs are nonselective cationic channels and conduct mainly Ca$^{2+}$, Na$^+$, and K$^+$. Two channels in this vast family are the exception, based on their restriction of Ca$^{2+}$. Indeed TRPM4 and TRPM5 do not differentiate between Na$^+$ and K$^+$ but are not permeable to divalent cations. When expressed in heterologous systems, these two channels show biophysical and regulatory properties similar to most NSCCa currents recorded in native cells. Thus this is now considered the molecular structure of the majority of these currents.

**TRPM4**

Since the publication of the first TRPM4 sequence in 2001 (92), several splice variants have been identified, called TRPM4a, b, and c in humans (92, 36, 52), differing in particular in their ionic selectivity. Although TRPM4b, the longest variant, has been shown to be expressed at the functional level in a variety of tissues, the relevance of the other variants remains unknown. We will therefore refer to TRPM4b as TRPM4 in the following sections. TRPM4 is a 1,214-amino acid molecule with six transmembrane domains encoded by a gene located on human chromosome 19 (36). Within its NH$_2$ and COOH terminal regions, the channel contains several PKC phosphorylation sites, two ATP-binding cassette transporter-like motifs, five calmodulin-binding sites, four Walker B sites (putative ATP-binding sites), a putative PiP$_2$ binding site, and a coiled-coil domain (see Ref. 85 for review).

The functional significance of most of these domains has been shown by electrophysiological recordings of the current elicited by heterologous expression of TRPM4 in HEK-293 cells. Single-channel currents have a linear conductance of ~25 pS with the higher open probability in the positive voltages, thus leading to the outward rectification observed in TRPM4 macroscopic currents (36). TRPM4 is selective for monovalent cations, with an order of permeability Na$^+$ > K$^+$ > Cs$^+$ > Li$^+$ and no permeability to Ca$^{2+}$. It is inhibited by intracellular free ATP in the 10 µM range, as well as by other adenine nucleotides (55). It is activated by internal Ca$^{2+}$ with a $K_D$ ranging from 0.4 (36) to 9.8 µM (53). This activation is modulated by ATP, calcium-calmodulin, and PKC (54). PiP$_2$ also regulates channel activity by modulating its calcium and voltage sensitivity (50, 93). Finally, heat modulates the voltage sensitivity of TRPM4, resulting in an increase in current (77).

Using molecular approaches, TRPM4 has been detected in a large numbers of tissues. In humans, it has been detected at high expression levels in the heart, pancreas, placenta, and prostate and at lower levels in the kidney, skeletal muscle, liver, intestines, thymus, and spleen (36, 52). It has been observed in several hematopoietic cell lines including T and B lymphocyte cell lines (Jurkat and Ramos) and the monocytic cell line U937 (35, 36).

TRPM5 shares ~50% amino acid sequence homology with TRPM4. Interestingly, these channels also share most of their biophysical and regulatory properties such as single-channel conductance, ionic selectivity, and voltage, calcium, PiP$_2$, and heat sensitivity (see Ref. 38 for review). However, TRPM5 is not inhibited by internal ATP (82) and is mainly expressed in taste receptor cells and the digestive tract (62). According to this remarkable difference in expression, TRPM4 is considered the molecular support for most NSCCa currents observed in native tissues.

In addition to molecular candidates, the world of NSCCa currents has also been waiting for pharmacological modulators. Unfortunately, the pharmacology for TRPM4 and TRPM5 is relatively unknown. The most commonly used blocker in physiological studies is flufenamic acid, which blocks both channels (82). Spermine also inhibits both channels (55), and the sulfonylurea glibenclamide inhibits TRPM4 (16). Unfortunately, these compounds are poorly selective and inhibit other kinds of ion channels. We have recently described two new modulators of TRPM4: TRPM4 is activated by the immunosuppressive pyrazole derivative BTP2 (76), and 9-phenanthrol, a hy-
droxytricyclic derivative, inhibits TRPM4 with no effect on TRPM5 (24).

With the discovery of the molecular structure of TRPM4 and the emergence of pharmacological compounds in the 2000s, our knowledge of the physiological roles of NSCCa currents has greatly improved. In the last few years, TRPM4 has been shown to regulate a surprising number of processes, as described in detail below. In short, under physiological conditions, after its activation by an increase in intracellular calcium, TRPM4 induces the entry of sodium, leading to membrane depolarization. TRPM4 is thus able to translate any internal calcium variation into a voltage variation, thereby modifying the driving force for the entry of ions, including Ca\textsuperscript{2+} itself (21, 36).

By modulating Ca\textsuperscript{2+} waves, TRPM4 regulates cytokine secretion in lymphocytes and insulin secretion in pancreatic \(\beta\)-cells (9, 35). It participates in mechanotransduction in cerebral arteries, by which vessel diameter is adapted to pressure (18, 19). It enhances neuronal excitability, promoting rhythmogenesis in the pre-Bötzinger complex (PBC) that controls breathing (15, 46). Finally, inherited mutations in TRPM4 lead to a conductance dysfunction in the human heart (34).

**Modulation of the Immune Response**

Increases in cytosolic free Ca\textsuperscript{2+} ([Ca\textsuperscript{2+}]\textsubscript{i}) are an ubiquitous signaling mechanism in hematopoietic cells such as lymphocytes, neutrophils, monocytes, and dendritic and mast cells. These [Ca\textsuperscript{2+}]\textsubscript{i} increases are implicated in diverse cellular functions, including cell adhesion, motility/chemotaxis, cell cycle progression, and apoptosis. Within the immune system, the intracellular Ca\textsuperscript{2+} influx induced by antigen receptors participates in both the development and maintenance of the immune response (39). On activation, Ca\textsuperscript{2+} originating from internal [mainly the endoplasmic reticulum (ER)] and external sources (through the plasma membrane) is delivered to localized areas of the cell at high concentrations. This rise in local [Ca\textsuperscript{2+}]\textsubscript{i} activates secondary signals that vary depending on their intracellular location, since the distribution of Ca\textsuperscript{2+}-sensitive signaling molecules (including channels) differs from one cellular subcompartment to another (plasma membrane vs. ER vs. mitochondria). The increase in [Ca\textsuperscript{2+}]\textsubscript{i} can display various patterns, such as a continuous rise, oscillations, and spikes, based on its amplitude as a function of time. The type of secondary signal activated also depends on the pattern of Ca\textsuperscript{2+} rise (17, 40).

Recently, the characterization of the molecular tandem of Stim1 (Stromal Interaction Molecule) and Orai1 has unveiled the mechanism responsible for Ca\textsuperscript{2+} entry through the CRAC current (20, 88). \(I_{\text{crac}}\), known as the store-operated Ca\textsuperscript{2+} current, is activated after Ca\textsuperscript{2+} depletion from the ER by the activation of the IP\textsubscript{3} receptor (see FIGURE 1). Subsequently, Ca\textsuperscript{2+} influx through the Orai1 channel activates the phosphatase calcineurin, which dephosphorylates the nuclear factor of activated T-cells (NFAT), thus enabling it to enter the nucleus and modulate gene transcription. This results in the production of several immune factors such as cytokines. Rather than a prolonged increase leading to loss of responsiveness (41), the Ca\textsuperscript{2+} signal in T-cells occurs in the form of oscillations, a critical process in differential gene expression (3).

The Na\textsuperscript{+} current supported by the TRPM4 channel has been revealed to be a key determinant in T-cell receptor-mediated Ca\textsuperscript{2+} waves. Indeed, the use of either a dominant negative form of TRPM4 and TRPM4-interference RNA in Jurkat T-cell lines leads to large intracellular calcium oscillations and interleukin-2 production in response to phytohemagglutinin stimulation (35). Furthermore, the facilitation of TRPM4 channel activity by BTP2 treatment decreases Ca\textsuperscript{2+} influx by depolarizing lymphocytes, resulting in the suppression of cytokine release (76). Thus TRPM4 activation at the peak of the Ca\textsuperscript{2+} wave leads to membrane depolarization, which decreases the driving force for Ca\textsuperscript{2+} entry. TRPM4 acts in concert with the K\textsuperscript{+} channels involved in membrane repolarization (6). Repolarization reestablishes the driving force for Ca\textsuperscript{2+} influx through \(I_{\text{crac}}\) so that the next oscillation in [Ca\textsuperscript{2+}]\textsubscript{i} can take place (see FIGURE 1).

TRPM4 also mediates the immune response upstream and downstream of T-cells. Dendritic cells (DC) are present in peripheral tissues, where they capture antigens. They undergo a process of maturation and migration to secondary lymphoid organs to reach and activate T-cells. In a TRPM4-deficient mouse model, we have shown that Ca\textsuperscript{2+} influx in DCs is increased after bacterial stimulation, leading to a migration defect (2). TRPM4 thus prevents Ca\textsuperscript{2+} overload to permit the proper homing of DCs to secondary lymphoid organs but has no effect on DC maturation, suggesting that the maturation and migration of DCs are regulated separately (2).

TRPM4 is also implicated in mast cell biology in the regulation of the allergic response. In sensitized hosts producing specific IgE molecules, secondary exposure to antigen initiates mast cell activation to release mediators, including histamine, resulting in vasodilatation and bronchoconstriction (45). The intracellular signal in mast cells is driven by Ca\textsuperscript{2+} influx through Orai1 channels (87). TRPM4-deficient mice exhibit an enhanced IgE-dependent mast cell activation. By modulating
the membrane potential of mast cells, TRPM4 reduces Ca\(^{2+}\) influx and thus decreases the allergic response (86).

Modulation of Insulin Secretion

Insulin is secreted by pancreatic \(\beta\)-cells of the islets of Langerhans and is involved in glucose uptake in tissues. Glucose itself promotes this secretion by a process involving several ion currents and the variation of intracellular calcium, as shown in FIGURE 2 (see Ref. 44 for review). Glucose enters the cell through specific transporters (GLUT) and is metabolized to produce ATP. The consecutive rise in ATP inhibits a \(K_{ATP}\) channel by interaction with a sulfonylurea receptor 1 (SUR-1), making room for depolarizing currents that activate voltage-dependent Ca\(^{2+}\) channels (VDCA). Ca\(^{2+}\) enters the cell by these channels, promoting the fusion of insulin-containing vesicles with the plasma membrane, leading to insulin release.

The inactivation of \(K_{ATP}\) channels is not sufficient to explain the shift in membrane potential in \(\beta\)-cells (1), and the TRPM4 channel is a good candidate for enhancing the glucose-induced membrane depolarization. In human \(\beta\)-cells, TRPM4 has been detected by Western blotting (9), and both transcripts and currents have been detected in pancreatic \(\beta\)-cell lines of other species including rats, mice, and hamsters (9, 42). In the rat pancreatic \(\beta\)-cell line INS-1, insulin secretion is reduced by the transfection of a dominant-negative form of TRPM4 (9). By its depolarizing effect, TRPM4 modulates VDCA activity and hence Ca\(^{2+}\) entry. Several pathways may initially modulate TRPM4 activity: 1) depolarization due to the closing of \(K_{ATP}\) channels by ATP, promoting TRPM4 activation; 2) \(Ca^{2+}\) release from the ER (4, 78); and 3) phosphorylation. It is known that PLC modulates insulin secretion (79). Thus the regulation of TRPM4 by the PLC-PKC pathway may be involved in this process. The dominant-negative form of TRPM4 reduces the insulin secretion induced by arginine-vasopressin (AVP), a Gq-coupled receptor agonist in \(\beta\)-cells (42). Under AVP stimulation, PLC hydrolyses PiP2 into IP\(_3\) and DAG. Although IP\(_3\) induces Ca\(^{2+}\)

![FIGURE 1. Modulation of the immune response in T-lymphocytes](http://physiologyonline.physiology.org/)

Antigen (Ag) presentation by dendritic cells and the major histocompatibility complex (MHC) induces T-cell receptor (TCR) activation coupled with the tyrosine kinase and phospholipase C (PLC) pathway, resulting in inositol 1,4,5-triphosphate (IP\(_3\)) formation. IP\(_3\) induces the depletion of calcium stores, leading to the opening of the Orai1 calcium-permeable channels. Calcium entry leads to a rise in intracellular calcium that activates calcineurin phosphatases, inducing the dephosphorylation of the nuclear factor of activated T-cells (NFAT). Once activated, NFAT enters the nucleus to modulate gene transcription and thus produce immune factors such as cytokines. Also, the rise in intracellular calcium activates K\(^+\) channels such as the calmodulin (CaM)-dependent K\(^+\) channel KCa3.1, which maintains the electrochemical gradient ($\Delta V$) in favor of Ca\(^{2+}\) entry. However, at high levels, Ca\(^{2+}\) activates TRPM4. The inward current through TRPM4 reduces the driving force for calcium entry and leads to a decrease in intracellular calcium by the Ca\(^{2+}\)-ATPase from the endoplasmic reticulum (SERCA) and the plasma membrane (PMCA). This causes the closing of TRPM4, resulting in the calcium waves necessary to avoid desensitization of the calcineurin/NFAT pathway during the immune response. DAG, diacylglycerol; ER, endoplasmic reticulum; IP\(_3\)R, IP\(_3\) receptor; PMCA, plasma membrane Ca\(^{2+}\)-ATPase; SERCA, sarco/endoplasmic reticulum Ca\(^{2+}\)-ATPase; STIM1, stromal interaction molecule 1; TRPM4, transient receptor potential melastatin 4; Tyr-K, tyrosine kinase.
release from the ER, DAG activates PKC, two processes known to increase TRPM4 activity, thereby favoring insulin secretion.

Insulin is secreted in a pulsatile pattern, following Ca\textsuperscript{2+} oscillations in the cell (79). Although the cell is depolarized by the closing of K\textsubscript{ATP} and the opening of TRPM4, promoting VDCa activation and Ca\textsuperscript{2+} entrance, a different process is necessary to reverse this phenomenon. The contribution of a large conductance Ca\textsuperscript{2+}-activated big K channel (BK\textsubscript{Ca}) has been shown in human β-cells (5). This channel responds to a rise in Ca\textsuperscript{2+} by generating a repolarizing current that favors the closing of the VDCa and TRPM4.

Interestingly, in β-cells, after stimulation with an agonist of the Ca\textsuperscript{2+} pathway, an activation of plasma membrane TRPM4 has been observed concomitant with the recruitment of new TRPM4 proteins contained in the membrane of Ca\textsuperscript{2+}-dependent exocytotic vesicles (9).

Mechanotransduction in the Cerebral Artery

Cerebral blood flow is maintained on a more or less constant scale by adjusting cerebrovascular resistance in response to changes in systemic pressure. A rise in pressure promotes the constriction of cerebral arteries. Myocyte contraction involves cell depolarization, which induces the opening of the VDCa and thus a rise in [Ca\textsuperscript{2+}]\textsubscript{i}. By interfering with proteins of the contractile network (calmodulin, myosin light chain kinase, etc.), Ca\textsuperscript{2+} induces arterial constriction. Once again, cell depolarization appears to be a link in the transduction signal. Recent studies on cerebral arterial myocytes have identified TRPM4 as a major actor in this depolarization (see FIGURE 3). The first evidence of this was obtained by the detection of TRPM4 mRNA and functional currents in rat vascular muscle cells (19). The downregulation of TRPM4 with antisense oligonucleotides reduces pressure-induced smooth muscle cell depolarization in isolated rat cerebral arteries (19). Furthermore, PKC stimulation facilitates TRPM4-induced depolarization and arterial constriction (18), in accordance with the PKC-dependent Ca\textsuperscript{2+} sensitization of TRPM4 (54).

The link between mechanical stress and TRPM4 activation is still unresolved. However, there is one potential mechanism: in rat cerebral arteries, the intracellular ryanodine receptor (RyR), which is involved in the stretch activation of TRPM4 (48), is

![FIGURE 2. Insulin secretion by pancreatic β-cells](image-url)

Glucose enters pancreatic β-cells through transporters (GLUT). The production of ATP by glucose hydrolysis in the mitochondria inhibits K\textsubscript{ATP} channels through interaction with SUR-1, resulting in cell depolarization. In addition, protein kinase C (PKC) potentiation of TRPM4 after Gq-coupled receptor stimulation and the activation of the PLC-DAG pathway leads to a depolarizing current. The resulting depolarization opens the voltage-dependent calcium channel (VDCa) to allow Ca\textsuperscript{2+} entry. The rise in intracellular Ca\textsuperscript{2+} promotes the fusion of insulin-containing vesicles with the plasma membrane but also stimulates Ca\textsuperscript{2+}-activated big potassium channel (BK\textsubscript{Ca}) channels, inducing cell repolarization. ∆V, membrane potential; K\textsubscript{ATP}, ATP-dependent potassium channel; PIP\textsubscript{2}, phosphatidylinositol 4,5-biphosphate; SUR-1, sulfonylurea receptor 1; V, vasopressin; V1R, vasopressin receptor type 1.
directly activated by membrane stretching or a Ca\(^{2+}\)-induced Ca\(^{2+}\) release (see Ref. 33 for review). TRPC6, a member of the TRP family, could be one of the channels involved in initial calcium entry (see FIGURE 3). Indeed, TRPC6 is Ca\(^{2+}\) permeable and activated by stretching by a mechanism involving a ligand-independent conformational switch of a Gq-coupled receptor by mechanical stress (see Ref. 68 for review). Furthermore, TRPC6 has been detected in the rat cerebral artery, and its downregulation by antisense oligonucleotides reduces pressure-induced smooth muscle cell depolarization and vasoconstriction of cerebral arteries, as for TRPM4 (91).

The physiological implications of this pathway have been shown in vivo in rats. The infusion of TRPM4 antisense oligonucleotides into the cerebrospinal fluid induces an elevation in mean arterial pressure, indicating an alteration of autoregulatory vasoconstrictor activity (67).

**Regulation of Respiratory Activity**

The generation of rhythmic inspiratory activity is a complex mechanism that needs both the intrinsic activity of specific neurons and synaptic contacts within the respiratory network (65). The PBC, localized in the brain stem, plays a critical role in inspiratory rhythmogenesis (73). We have shown that rat brain stem neurons exhibit a spontaneous rhythmic bursting activity after a few days in primary culture, concomitant with the development of synaptic connections (26). These essential connections are the reason why most studies describing respiratory rhythm generation have been carried out in slice preparations from the PBC.

An NSC\(_{\text{Ca}}\) current with the specific characteristics of both TRPM4 and TRPM5 has been identified in mouse brain stem slices containing the PBC (46). However, channel inhibition by ATP points more to the TRPM4 channel than to TRPM5 (46). The inhibition of this current by flufenamic acid reduces burst generation. Thus TRPM4 might be a regulator of the respiratory rhythm (15, 61). Membrane depolarization induced by the TRPM4 current may help the cell reach the threshold for voltage-gated Na\(^{+}\) channel activation, leading to an action potential. In accordance with this idea, the TRPM4 modulator PiP\(_2\) has been shown to regulate inspiratory burst activity by modulating NSC\(_{\text{Ca}}\) currents in the PBC (13).

Synaptic glutamatergic transmission plays a crucial role in the control of respiratory rhythmicity. The blockade of both N-methyl-D-aspartate (NMDA) and non-NMDA glutamate receptors in the PBC causes alterations in rhythmic activity (49). Although NMDA receptors do not modulate TRPM4 to a great extent in the PBC (57), both AMPA and metabotropic glutamate receptors contribute to TRPM4 activation (56, 57). The activation of the former leads to a depolarizing current capable of opening the VDCa, whereas the latter triggers the PLC pathway, inducing IP\(_3\) production, which allows the liberation of Ca\(^{2+}\) from the ER. Thus both receptors lead to a rise in cytoplasmic Ca\(^{2+}\), favoring the activation of TRPM4. Therefore, TRPM4, probably in combination with other depolarizing currents from the VDCa.

![FIGURE 3. Stretch-induced constriction of cerebral arteries](http://physiologyonline.physiology.org/)

Membrane stretching stimulates Gq-coupled receptors that promote TRPC6 activation. Calcium entry, perhaps in addition to the release of calcium from the sarcoplasmic reticulum (SR) after ryanodine receptor (RyR) stimulation, activates TRPM4. This activation leads to cell depolarization, promoting VDCa opening and thus the entry of calcium. Calcium, in combination with calmodulin, activates the myosin light-chain kinase (MLCK), resulting in myocyte contraction. In these cells, PKC also promotes TRPM4 activation. \(\Delta V\), membrane potential; GPCR, G-protein coupled receptor; TRPC6, transient receptor potential canonical 6.
and AMPA receptors, leads to an excitatory post-synaptic depolarization that triggers bursting activity, as summarized in Figure 4.

Unfortunately, since no data are available either with regard to the use of interference RNA or a TRPM4-deficient mouse model, and despite the ATP-blocking data observed by Mironov (46), the final validation of the TRPM4 channel as the molecular counterpart of the NSCCa current in the PBC is still missing.

TRPM4 in the Heart

A TRPM4 mutation has been reported in patients with an inherited cardiac disorder. The substitution of glutamate in position 7 by lysine leads to a progressive blockade of the conduction through the cardiac bundle branch (34). This is the first mutation in TRPM4 to be correlated with a pathophysiological role for this channel in humans. Using heterologous expression, the authors show that the mutation does not modify the biophysical properties of the channel, but the altered SUMOylation of the mutant protein results in an increase in protein and current levels at the plasma membrane. However, the link between the gain of function and the conduction block remains puzzling. By decreasing membrane potential, TRPM4 may alter the availability of the voltage-dependent Na\(^+\) channel and thus impair cardiac conduction (31, 64).

TRPM4 currents have been detected in sinoatrial node cells (16) and atrial cardiomyocytes (25), but at very low levels in ventricular cardiomyocytes (28). This pattern of expression is altered by ventricular hypertrophy. Indeed, we have observed an overexpression of TRPM4 in ventricular cardiomyocytes from spontaneously hypertensive rats that develop cardiac hypertrophy associated with ventricular arrhythmias (28). We hypothesize that TRPM4 participates in a calcium-activated transient inward current (\(I_{\text{IN}}\)) known to be responsible for the delayed after-depolarizations observed in hypertrophied ventricles (see Ref. 27 for review).

Perspectives

A profusion of additional data predicts the discovery of other physiological implications for TRPM4. Several tissues express TRPM4, although its physiological relevance is still to be decrypted. TRPM4 mRNA is detected at high levels in the human and rat prostate (22, 90). Its functional distribution along the renal tubules (10, 11) may reveal its involvement in regulating renal reabsorption by modulating the driving force for ion entry in response to Gq-coupled receptors or variations in pH.

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**FIGURE 4. Respiratory rhythm regulation in the pre-Bötzinger complex**

Glutamate release from the presynaptic neuron stimulates both mGLU and \(\alpha\)-amino-3-hydroxyl-5-methyl-4-isoxalone-propionate receptor receptors (AMPA-R) on the postsynaptic neuron. Na\(^+\) entry through AMPA-R depolarizes the cell, inducing VDCa activation. Metabotropic-glutamate receptor (mGLU-R) activation triggers PLC pathway activation, leading to \(I_{\text{P2}}\) production, which in turn stimulates \(I_{\text{P3}}\) receptors on the endoplasmic reticulum. The activation of both VDCa and \(I_{\text{P3}}\) results in the rise of intracellular calcium, favoring TRPM4 activation and thus cell depolarization. In conjunction with AMPA-R and VDCa, TRPM4 induces an excitatory postsynaptic potential that activates voltage-dependent sodium channel (VDNa) channels to induce bursting activity. EPSP, excitatory postsynaptic potential; ER, endoplasmic reticulum; PIP\(_2\), phosphatidylinositol 4,5-biphosphate.
In addition, the pathological implications of TRPM4 are only now emerging. TRPM4 is expressed in the endothelium (74), including in brain capillaries (14). In this tissue, it has recently been shown that the de novo expression of TRPM4 following spinal cord injury is associated with capillary fragmentation leading to secondary hemorrhage (23). This phenomenon does not occur in rats treated with TRPM4 antisense mRNA and in a TRPM4-deficient mouse model.

There are intriguing data regarding the interaction between TRPM4 and other membrane proteins. When heterologously expressed, TRPM4 modulates store-calculated calcium entry through physical interaction with TRPC3 (58). An ATP-inhibited NSC1a channel interacts with the SUR-1 receptor in astrocytes (7, 71). Despite differences, it cannot be totally ruled out that this channel corresponds to TRPM4 (72). If confirmed, these interactions may lead to new perspectives in the comprehension of TRPM4 regulation and its physiological impact.

The complex example of immune cells illustrates the large spectrum of TRPM4 actions. Since it responds to and modulates intracellular Ca2+ concentrations, its impact on cell signaling is diffuse across many tissues.

REFERENCES


