Chapter 42
TRP Channels of Islets

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Abstract In the normal human body pancreatic β-cells spend most of the time in a READY mode rather than in an OFF mode. When in the READY mode, normal β-cells can be easily SWITCHED ON by a variety of apparently trivial stimuli. In the READY mode β-cells are highly excitable because of their high input resistance. A variety of small depolarizing currents mediated through a variety of cation channels triggered by a variety of chemical and physical stimuli can SWITCH ON the cells. Several polymodal ion channels belonging to the transient receptor potential (TRP) family may mediate the depolarizing currents necessary to shift the β-cells from the READY mode to the ON mode. Thanks to the TRP channels, we now know that the Ca^{2+}-activated monovalent cation selective channel described by Sturgess et al. in 1986 (FEBS Lett 208:397–400) is TRPM4, and that the H_2O_2-activate non-selective cation channel described by Herson and Ashford, in 1997 (J Physiol 501:59–66) is TRPM2. Glucose metabolism generates heat which appears to be a second messenger sensed by the temperature-sensitive TRP channels like the TRPM2 channel. Global knock-out of TRPM5 channel impairs insulin secretion in mice. Other TRPs that may be involved in the regulation of β-cell function include TRPC1, TRPC4, TRPM3, TRPV2 and TRPV4. Future research needs to be intensified to study the molecular regulation of the TRP channels of islets, and to elucidate their roles in the regulation of human β-cell function, in the context of pathogenesis of human islet failure.

42.1 Introduction

The pancreatic β-cells are sensors for glucose, amino acids, fatty acids as well as for incretins, other hormones, neurotransmitters and growth factors [1]. Many ion channels, G-protein coupled receptors, and other plasma membrane and intracellular receptors are involved in triggering different sets of interconnected signaling events

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M.S. Islam (ed.), *Transient Receptor Potential Channels*, Advances in Experimental Medicine and Biology 704, DOI 10.1007/978-94-007-0265-3_42,
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that regulate exocytosis of insulin in response to a variety of stimuli. From in vitro experiments where glucose has been used as the only active agent, it is known that two important events occur when glucose stimulates insulin secretion. These are depolarization of the membrane potential and an increase in the cytoplasmic free Ca^{2+} concentration ($[Ca^{2+}]_i$), often in the form of oscillations [2]. A handful of ion channels are known to be involved in mediating these two interdependent events [3]. Among them, the most extensively studied one is the ATP-sensitive K^+ (K_{ATP}) channel. β-cells and some cells in the hypothalamus are the only cells in the body that use ATP as a cytoplasmic second messenger; the K_{ATP} channels being the sensors for this unique second messenger. These channels are targets for some widely prescribed anti-diabetic drugs. Mutations of K_{ATP} channel can cause congenital hyperinsulinism or neonatal diabetes in human [4]. Not surprisingly, K_{ATP} channels hugely dominate the field of islet research; cellular processes are defined in terms of this channel, and are branded either "K_{ATP}-channel-dependent" or "K_{ATP}-channel-*in*dependent". This scenario, including the use of the term "consensus model" leaves little room to accommodate the TRP channels in the existing model(s) of stimulus secretion coupling in β-cells.

It is worthwhile to consider a slightly different conceptual scenario where one could possibly implicate the TRP channels in the regulation of insulin secretion. Human β-cells secrete insulin all the time both during the fasting state and during the fed state. Insulin can cause potentially life-threatening hypoglycemia. As a safety device, nature has equipped these cells with K_{ATP} channels which can rapidly switch OFF insulin secretion to a great extent, when hypoglycemia is imminent. In most in vitro experiments, where relatively simple physiological solutions containing < 3 mM glucose are used as the sole nutrient, β-cells are in OFF mode [2]. In the OFF mode, the cytoplasmic free Ca^{2+} concentration ($[Ca^{2+}]_i$) is stable around 50–100 nM [2]. In vivo, β-cells are unlikely to be in the OFF mode even after overnight fasting because they are continuously bathed in many nutrients and hormones in addition to about 5 mM glucose. In fact, β-cells secrete insulin, albeit at low level, even during hypoglycemia, which is corrected by counter-regulatory hormones and gluconeogenesis. In their native environment, β-cells probably spend most of the time at least in a READY mode rather than in the OFF mode. In the READY mode, the degree of readiness or excitability of β-cells depends upon the input-resistance of the plasma membrane which in turn depends mostly on the degree of closure of the K_{ATP} channels and the magnitude of the background depolarizing currents. When in the READY mode, normal β-cells can be easily switched ON by a variety of apparently trivial stimuli acting alone or in combination. A variety of small depolarizing currents mediated through a variety of cation channels triggered by a variety of chemical and physical stimuli can switch ON these cells. The molecular basis of such depolarizing currents remained elusive for more than two decades. After the discovery of the TRP channels, their roles in β-cell was not extensively studied partly because of the scenario described in the previous paragraph, and partly because of the lack of specific pharmacological tools, specific antibodies and lack of obvious phenotypes in knock-out mouse models.

TRP channels of β-cells have been described in a recent review [2]. In this chapter, I shall sum up the background information and elaborate some of the recent developments in the field and give my own views and speculations. One note of caution is that research in cell and molecular biology is neither randomized, nor double-blinded, and seldom adequately controlled. The huge pressure on scientists for publication, and lack of adequate regulatory mechanisms for controlling accuracy of data make it inevitable that some enthusiasts can reveal what is suggestive and hide what is vital. Nevertheless, the review is based on a handful of published papers on the topic assuming that all published reports are of high scientific quality.

It is becoming increasingly evident that β-cells have many TRP channels (Table 42.1), and that some of these channels can contribute to membrane depolarization, Ca^{2+} signaling, insulin secretion and cell survival. I shall start with the TRPM4 and TRPM5 because these two are pretty hot at the time of this writing.

Table 42.1 TRP channels of insulin-secreting cells

Channel	Cell type	Methods	Reference
TRPC1	MIN6, mouse islet	RT-PCR, NB	[5, 6]
	INS-1, rat β-cells	RT-PCR	[7]
TRPC2	MIN6, mouse islet	RT-PCR	[6]
TRPC3	Mouse islets	RT-PCR	[6]
TRPC4	MIN6, βTC3, INS-1		
	rat β-cells	RT-PCR [7], NB [6]	[6, 7]
TRPC5	βTC3	RT-PCR	[6]
TRPC6	MIN6	RT-PCR	[6]
TRPM2	Human islets	RT-PCR [8], WB [9]	[8, 9]
	INS-1E	EP	[9]
	RIN-5F	EP, IF [10], WB [10]	[10, 11]
	CR1-G1	EP, RT-PCR	[12]
	HIT-T15	EP	[13]
	Mouse β-cells	IF [11], EP [14]	[11, 14]
	Rat β-cells	IF	[11]
TRPM3	INS-1, mouse islets	EP, RT-PCR, NB, WB	[15]
	Mouse β-cells	EP	[15]
TRPM4	INS-1, RINm5F	RT-PCR, IP, EP	[16]
	HIT-T15		
	MIN6, HIT-T15, RINm5F		
	β-TC3, INR1G9	EP	[17]
	Human β-cells	IF	[17]
TRPM5	MIN6, INS-1		
	Human islets	RT-PCR	[18, 19]
	Mouse β-cells	RT-PCR, EP, IF	[19]
TRPV1	INS-1, RINm5F, rat islets	RT-PCR, WB, IF	[20]
TRPV2	MIN6	RT-PCR, IB	[21]
	Mouse β-cells	IF	[21]
TRPV4	MIN6	RT-PCR, Ca^{2+} imaging	[22]

EP, electrophysiology; IF, immunofluorescence; IB, immunoblot; NB, Northern blot; WB, Western blot

42.2 TRPM4 and TRPM5

TRPM4 and TRPM5 have been reviewed in Chapter 8 by Romain Guinamard, Laurent Sallé, and Christophe Simard, in this book. These channels are activated by an elevated $[Ca^{2+}]_i$, and later on desensitized to further activation by Ca^{2+}. They are permeable to monovalent cations but almost impermeable to the divalent ones. It is speculated that TRPM4 and/or TRPM5, and possibly heterotetramers of TRPM4/TRPM5 may account for some components of the Ca^{2+} activated non-selective cation currents (Ca-NS) described in β-cells in some earlier reports [6, 23–25].

TRPM4 is inhibited by adenine nucleotides whereas TRPM5 is not, allowing one to distinguish between these two currents. The earliest indication for the presence of a TRPM4-like current in β-cells can be tracked back to the studies of Sturgess et al., reported in 1986 [25]. They described in CRI-Gl rat insulinoma cells a ~25 pS nonselective cation current (NSCC) activated by Ca^{2+} and inhibited by adenine nucleotides. The potencies of different adenine derivatives for the inhibition of this current (AMP > ADP > ATP > adenosine) are slightly different from those reported for the cloned TRPM4 over-expressed in heterologous cells (ADP > ATP > AMP >> adenosine) [26]. More than a decade later, Leech and Habener described in HIT-T15 cells, a ~25–30 pS Ca^{2+}-activated NSCC that was inhibited by ATP. Their results suggest the possibility that they were perhaps dealing with the TRPM4 current [24]. However, the linear current voltage (*I/V*) relationship of their current is not typical for TRPM4 or TRPM5, which usually shows activation at positive membrane potentials [27].

TRPM4 currents have now been described in a variety of rodent insulinoma and glucagonoma cells. In these cells, an elevated $[Ca^{2+}]_i$ activates the channel with an EC_{50} of ~0.57–1.25 μM [17]. Open probability of TRPM4 increases also upon membrane depolarization but only if $[Ca^{2+}]_i$ is sufficiently high. The TRPM4 current activated by an increase of $[Ca^{2+}]_i$ is biphasic where the first phase develops within seconds and the second phase develops slowly. Development of the second phase is thought to be due to incorporation of TRPM4 channels into the plasma membrane as a result of exocytosis. Inhibition of endogenous TRPM4 by a truncated dominant negative construct of TRPM4 reduces the magnitude of Ca^{2+} signal and insulin secretion in response to glucose or agonists of receptors coupled to phosphoinositide-specific phospholipase C (PI-PLC) [17]. So far, TRPM4 current has not been described in primary β-cells. However, TRPM4 protein can be detected by immunofluorescence in human β-cells [17]. Several splice variants of TRPM4 are known and of them TRPM4b (often referred to simply as TRPM4) is the one most studied. In TRPM4 knockout mice insulin secretion is normal possibly because of the presence of the related TRPM5 channel [28].

Amino acid sequence of TRPM4 shows two stretches that look like signature motifs of ATP binding cassette proteins (ABC transporters), and four nucleotide binding domains (NBD). Consistent with this, TRPM4 is inhibited by glibenclamide at least in some tissues [29]. The adenine nucleotide sensitive Ca^{2+}-activated NSCC described by Sturgess et al., is not inhibited by tolbutamide or glibenclamide [30]. As mentioned earlier, TRPM4 is directly inhibited by cytoplasmic ATP and

other adenine nucleotides without requiring Mg^{2+} or hydrolysis of the nucleotides. However, ATP also inhibits Ca^{2+}-desensitization of TRPM4 [31]. TRPM4 has several putative phosphorylation sites for PKA and PKC. It also has arginine lysine rich sequences that are putative binding sites for phosphatidylinositol-4,5-bisphosphate (PIP2). Two well known regulators of TRPM4 are PKC and PIP2. The Ca^{2+}-sensitivity of TRPM4 is enhanced by PKC phosphorylation [31]. This is interesting given that different PKC isoforms regulate diverse functions of β-cells [32]. PIP2 shifts the voltage activation curve of TRPM4 towards negative voltages and also prevents Ca^{2+}-mediated desensitization of the channel; depletion of PIP2 inhibits the channel [33]. This mode of regulation may be relevant for β-cells since glucose, by way of altering the cytoplasmic ATP-to-ADP ratio, alters the PIP2 concentration in the plasma membrane in an oscillatory fashion [34].

Compared to TRPM4 which is expressed in a wide variety of tissues, the expression of TRPM5 is more restricted [35]. The latter channel is abundant in taste bud, intestine, pancreas and pituitary [35]. In fact, TRPM5 is best known for its role in taste signaling [36]. TRPM5 mRNA is detected in MIN6 cells [18] [but see [37]], INS-1 cells, purified mouse β-cells and human islets [18, 19]. By immunofluorescence, TRPM5 protein is detected in the β-cells of wild type mice and not in those of the knock-out mice [19].

Human TRPM4 and TRPM5 channels are activated by $[Ca^{2+}]_i$ with nearly similar EC_{50} values (~ 840–885 nM) [18]. Mouse TRPM4 is much less sensitive to $[Ca^{2+}]_i$ (EC_{50} of ~ 2,000 nM) compared to the mouse TRPM5, which is highly sensitive to $[Ca^{2+}]_i$ (EC_{50} of ~ 700 nM) [38]. Human TRPM5 is rapidly inhibited by high $[Ca^{2+}]_i$ (IC_{50} of 1 μM) (18), whereas mouse TRPM5 is not inhibited by high $[Ca^{2+}]_i$ (18). Activation of human TRPM5 depends more on the rate of rise of $[Ca^{2+}]_i$ rather than the magnitude of $[Ca^{2+}]_i$ [18]. In β-cells Ca^{2+} induced Ca^{2+} release (CICR) causes fast and transient increase of $[Ca^{2+}]_i$ [39, 40], and my speculation is that CICR is particularly suitable for activation of TRPM5 in human β-cells. Activation of mouse TRPM5 and TRPM4 depends on the magnitude of $[Ca^{2+}]_i$ and not on the rate of increase of $[Ca^{2+}]_i$ [38].

42.2.1 Role of TRPM4 and TRPM5 in Stimulus-Secretion Coupling in β-Cells

TRPM5 turns out to be essential for stimulation of insulin secretion by glucose in mouse. Knock-out of the channel reduces [19] or abolishes [41] glucose-induced insulin secretion. Colsoul et al., demonstrate that fast oscillations in membrane potential and corresponding fast oscillations in $[Ca^{2+}]_i$ that are induced by glucose in normal mouse islets are almost completely lost when TRPM5 is knocked-out [19]. It appears that glucose alone can successfully shift β-cells of TRPM5 knock-out mouse from the OFF mode to the READY mode, but fails to shift to the ON mode. In the READY mode, β-cells of TRPM5 knock-out mouse display slow oscillations in membrane potential and corresponding slow oscillations in $[Ca^{2+}]_i$, and the cells secrete insulin at low level [19]. Apparently, shifting from the READY

mode to the ON mode requires, in this instance, functional TRPM5 channels. Furthermore, it is apparent that even small depolarizing currents are enough to shift the β-cells from the READY mode to the ON mode. For instance, TRPM5 current described by Colsoul et al., is of small magnitude (~ 20 pA at –80 mV under maximal stimulation by Ca^{2+}) [19]. Perhaps one electrophysiological fingerprint of the ON mode in mouse islets is the fast oscillations in membrane potential and corresponding fast oscillations in the $[Ca^{2+}]_i$.

Potential factors that can link stimulation of β-cells by glucose to the activation of the TRPM5 channels include 1. glucose-induced increase in $[Ca^{2+}]_i$; 2. glucose-induced increase in the membrane potential [42]; 3. glucose-induced increase in the concentration of cytoplasmic arachidonic acid [43, 44]; and 4. glucose-induced increase in the concentration of PIP2 [34, 36]. It is noteworthy that mouse β-cells express the G-protein coupled heterodimeric sweet taste receptor T1R2/T1R3, and that even noncaloric sweet agents can stimulate insulin secretion in a Na^+-dependent manner [37]. Glucose may possibly directly activate these sweet taste receptors in the β-cells and thereby activate the TRPM5 channel as is the case in the taste cells [36].

TRPM5 takes part in mediating insulin secretion even when glucose is used as the sole agonist. It is however tempting to speculate that both TRPM4 and TRPM5 may also participate in the regulation of insulin secretion by different incretins, neurotransmitters and hormones. For instance, GLP-1 increases cytoplasmic $[Na^+]$ [45] by activating a Ca^{2+}-activated non-selective cation current mainly carried by Na^+ and blocked by La^{3+} [23]. It has been reported that GLP-1 activates a current that is mimicked by mitotoxin [46]. However, it is not known whether maitotoxin activates any TRP channels. There are some indirect evidence that mitotoxin activates TRPC1 [47] and β-cells have TRPC1 [5]. On the other hand, it has been demonstrated that maitotoxin binds to the plasma membrane Ca^{2+} ATPase and converts it into a Ca^{2+}-permeable non-selective cation channel [48].

In normal human body, insulin secretion ensues almost immediately in connection with the ingestion or even anticipation of food. Such preabsorptive phase of insulin secretion is subdivided into a cephalic phase and an enteric phase. In reality, these phases of insulin secretion merge with one another to a variable extent. One can speculate that in the cephalic phase of insulin secretion $[Ca^{2+}]_i$ increases in β-cells because of the actions of neurotransmitters like acetylcholine released from the vagus nerve. Such increase of $[Ca^{2+}]_i$ in turn activates TRPM4 and TRPM5 leading to membrane depolarization. In this scenario, TRPM4 and TRPM5 may play a key role in shifting the β-cells from the READY mode to the ON mode. Thus, TRPM4 and TRPM5 provide a distinct mechanism for coupling $[Ca^{2+}]_i$ to membrane depolarization. It is possible that an increase of $[Ca^{2+}]_i$ by a variety of mechanisms, for instance, by CICR, IP3, NAADP, or cADPR may increase or sustain depolarization through the activation of TRPM4 and TRPM5 channels. It may be noted that TRPM5 affects insulin secretion not just by way of membrane depolarization. For instance, islets of TRPM5 knock-out mice fail to secret insulin even when they are persistently depolarized by arginine, suggesting that a distinct type of oscillations in membrane potential i.e. the fast oscillations are necessary for optimal insulin secretion [41].

42.3 TRPM2 (Formerly Called LTRPC2)

42.3.1 TRPM2 and β-Cells

In 1994, Reale et al., described a non-selective Ca^{2+} permeable cation channel activated by β-NAD^{+} and inhibited by AMP in CRI-G1 insulinoma cells [49]. Later on, the same group reported that this channel is activated by H_2O_2 [50]. The currents described in these papers have some key features of the TRPM2 current e.g. linear current-voltage relationship, extremely long single channel open times, and the requirement of intracellular Ca^{2+} for activation of the current by H_2O_2 [50, 51]. Today we know that TRPM2 channels are present not only in the rodent insulinoma cells but also in the primary β-cells [14]. The most potent and specific endogenous activator of TRPM2 is cytoplasmic ADP ribose (ADPR) that binds to the NUDT9-H domain which acts as an enzyme (ADPR hydrolase) [52]. ADP ribose formed by degradation of NAD^{+} by poly(ADP ribose) polymerase activates TRPM2. H_2O_2 does not have any direct effect on the TRPM2 channel; in stead it activates TRPM2 by increasing the concentration of cytoplasmic free ADPR [53]. In primary mouse β-cells, ADPR activates a plasma membrane current characteristic of TRPM2, whereas it fails to do so in the β-cells from the TRPM2 knock out mouse [14]. It is likely that human β-cells also express TRPM2. In these cells, H_2O_2 activates Ca^{2+} entry through the plasma membrane [9]. By Western blot two isoforms of TRPM2 protein can be detected in the membrane fraction obtained from whole human islets [9]. These are the full length or the long form of the channel (TRPM2-L), and a short form of the channel (TRPM2-S) where the four C-terminal transmembrane domains, the putative pore region and the entire C-terminus are truncated [54].

To study the distribution of TRPM2 in different cells of islets, we performed immunohistochemistry of formalin fixed, paraffin embedded human pancreas. We used two antibodies: 1. an affinity purified rabbit polyclonal IgG directed against an epitope on the N-terminal part of TRPM2 (anti-TRPM2-N) (BL 970. Cat. no. A300-414A, Bethyl laboratories Inc., USA). 2. an affinity purified rabbit polyclonal IgG directed against the C-terminal part of the TRPM2 (anti TRPM2-C) (BL969, Cat. no.A300-413A, Bethyl laboratories Inc.). The immunogen for anti-TRPM2-N was the peptide ILKELSKEEEDTDSSEEMLA, which represents the amino acids 658–677 of human TRPM2 encoded within exon 13. The immunogen for anti-TRPM2-C was the peptide KAAEEPDAEPGGRKKTEEPGDS, which represents amino acids 1,216–1,237 of human TRPM2 encoded within exon 25. However, from the Western blot and from immunohistochemistry, we concluded that these antibodies are not suitable for use in immunohistochemistry.

42.3.2 Role of TRPM2 Channel in Stimulus-Secretion Coupling in β-Cells

Like TRPM4 and TRPM5, TRPM2 is also a Ca^{2+}-activated non-selective cation channel [13, 55], but unlike TRPM4 and TRPM5, TRPM2 is permeable to Ca^{2+}.

TRPM2 current is mainly carried by Na^+. The permeability ratio p_{Ca}:$p_{Cs,}$ as estimated from shifts in reversal potentials is low (~0.54 in CRI-G1 insulinoma cells) [12]. Nevertheless, activation of the TRPM2 channel increases $[Ca^{2+}]_i$ [9]. Surprisingly, the permeability of TRPM2 for Ca^{2+} increases (p_{Ca}:p_{Na} = 5.83), when the current is activated by heat [11]. Activation of human TRPM2 by ADPR requires Ca^{2+}. Ca^{2+} activates all isoforms of TRPM2, including the ones that are unable to bind ADPR [13]. The Ca^{2+} binding sites are located in a restricted space intracellularly but in the immediate vicinity of the pore region [56]. Thus extracellular Ca^{2+} may be the major source of Ca^{2+} for the activation of TRPM2. In the presence of stable low micromolar concentration of cytoplasmic ADPR, TRPM2 essentially behaves like a Ca^{2+}-activated channel [56, 57]. Perhaps this mode of regulation enables TRPM2 mediate its physiological function in stimulus-secretion coupling in the β-cells. Extracellular Ca^{2+} that enters through the TRPM2 channel can activate the channel by binding to the activation sites that lie near the pore entrance of the channel [56]. This is a potential mechanism for prolongation of activation of TRPM2 in a self sustained manner [56]. An increase of $[Ca^{2+}]_i$ upon stimulation of PI-PLC linked receptors is also able to activate TRPM2. For instance, activation of muscarinic acetylcholine receptor in hamster insulinoma HIT T15 cells activates TRPM2 channel [13]. Thus, TRPM2 provides another mechanism for linking $[Ca^{2+}]_i$ increase to membrane depolarization as has been described for TRPM4 and TRPM5 in a previous paragraph.

From whole cell studies it appears that TRPM2 channel is activated by many agents some of which act synergistically. Some of these agents act directly on the TRPM2 channel and others act indirectly. Effects of ADPR, Ca^{2+}, NAADP and nicotinic acid adenine dinucleotide (NAAD) are direct and their effects can be demonstrated in inside-out patches. NAADP is a low affinity partial agonist and is thus unlikely to be a physiologically relevant activator of TRPM2 [53]. TRPM2 could possibly provide a mechanism whereby mitochondrial reactive oxygen species (ROS) couple glucose metabolism to insulin secretion [58, 59]. However, in intact cells TRPM2 is activated by ROS only indirectly by increasing the concentration of cytoplasmic free ADPR as a result of oxidative/nitrosative stress [53]. It was reported that one isofom of TRPM2 e.g. TRPM2-ΔC is not activated by ADPR, but is activated by H_2O_2 suggesting that ROS can directly activate the channel [60]. However, a later study could not reproduce this finding [61]. "Activation" of TRPM2 by β-NAD^+ and cyclic ADP ribose (cADPR) is actually due to the contamination of these substances with ADPR [53, 57]. β-NAD^+ may have some direct effects comparable to the effects of NAAD. In intact cells, high concentrations of cADPR potentiate ADPR-incuced activation of TRPM2 [62] but such high concentrations of cADPR do not occur in cells. According to Togashi et all cADPR couples glucose metabolism to the activation of TRPM2. They have used high concentrations of cADPR (up to 100 μM). Such concentrations of cADPR have nothing to do with physiology [11]. They used cADPR from Sigma and did not comment on the purity of cADPR [11]. It should be noted that some lots of cADPR may contain as much as 25–50% ADPR as contaminant [53, 57, 62]. I suspect that many effects of cADPR published in many papers are due to the use of impure cADPR.

Other modulators of TRPM2 activity include arachidonic acid, PKA, and heat. In β-cells, arachidonic acid is produced upon glucose metabolism and it has been demonstrated that arachidonic acid can stimulate TRPM2 via a specific binding domain [10]. TRPM2 channel activity is potentiated by PKA phosphorylation [11]. Consistent with this, stimulation of insulin secretion by GLP-1 analogue Exendin-4 is inhibited by siTRPM2 [11]. GLP-1 also produces NAADP and cADPR but as mentioned before, these agents can positively modulate TRPM2 channel activity only when they are used in high concentrations [63]. TRPM2 knock-out mice have impaired insulin secretion in response to glucose and GLP-1[64]. In human, at least three TRPM2 gene variants are associated with reduced insulin secretion as estimated by homeostatic model assessment [65].

It is possible that various insulinotropic agents may engage TRPM2 by producing a multitude of factors like Ca^{2+}, heat, ROS, arachidonic acid, cADPR, and NAADP. The channel, thus, may act as a coincidence detector and even though increases in any of these factors alone are small or absent, the net effect of such increases on the TRPM2 channel can be large. This is however a pure speculation.

ADPR-induced activation of TRPM2 is inhibited by AMP (IC_{50} ~ 10 μM) and cADPR-induced activation of TRPM2 in intact cells is inhibited by 8-Bromo-cADPR [62]. However effects of these inhibitors are likely to be indirect [53]. It is not known whether inhibition by AMP is via the AMP-kinase. Among the pharmacological inhibitors of TRPM2, we have found N-(p-amylcinnamoyl) anthranilic acid (ACA) a useful one but it does inhibit other TRP channels e.g. TRPM8 and TRPC6 to a variable extent [66]. ACA is also an inhibitor of phospholipase A2 [9]. It is unclear whether the inhibitory effect of ACA on TRPM2 is a direct one or an indirect one via phospholipase A2.

42.3.3 Heat as a Physical Second Messenger

The ionic flux through many channels is sensitive to temperature (typical Q_{10} values 1.2–4), probably because of temperature-dependence of diffusion. However, TRPM2 is specialized to detect heat ($Q_{10} = 15.6$), the temperature threshold and the temperatures for optimal activity being ~34°C and ~37°C respectively [11]. In the presence of ADPR, the Q_{10} value is as high as 44 [11]. At normal body temperature TRPM2 of β-cells is constitutively active possibly contributing to the well known background depolarizing current. It is plausible that nutrient metabolism increases local temperature of individual β-cells [67] leading to further activation of the TRPM2 channel leading to membrane depolarization. In this scenario, heat could be seen as a physical second messenger for stimulus-secretion coupling in the β-cells. Thus, TRPM2 provides a molecular basis for the well known steep temperature dependence of insulin secretion, which is not the case for secretion of other hormones like glucagon from the α-cells or catecholamines from the chromaffin cells [68].

42.3.4 TRPM2 and β-Cells Death

Being a Ca^{2+} permeable redox sensitive channel, TRPM2 confers susceptibility to cell death caused by oxidative stress and consequent perturbation of Ca^{2+} homeostasis. Under conditions of oxidative stress, the concentration of cytoplasmic ADPR is high. Such high concentration of ADPR, together with high concentration of $[Ca^{2+}]_i$ synergize to cause massive Ca^{2+} influx and Ca^{2+} overload. Thus, in RIN-5F cells, H_2O_2 and TNFα -induce cell death which can be suppressed by treatment with antisense TRPM2 [10]. Alloxan-induced oxidative stress can cause β-cell death by many mechanisms and one of these may be the activation of TRPM2 [50]. One short isoform of TRPM2 (TRPM2-S) does not form a channel; in stead, it inhibits the activity of the TRPM2-L and thereby inhibit cell death [54, 69]. It is possible that the ratio of these two isoforms of TRPM2 channels in human β-cell may determine the degree of susceptibility or resistance of these cells to oxidative stress. Human β-cells are resistant to alloxan and such resistance has been attributed to the fact that human β-cells express only low level of the glucose transporter GLUT2 which transport alloxan into the β-cells [70–72]. However, since TRPM2 mediates alloxan-induced Ca^{2+} overload [50], it can be speculated that resistance of human β-cells to alloxan could partly be attributed to the inhibitory action of the TRPM2-S isoform expressed in these cells.

42.3.5 TRPM2 as an Intracellular Ca^{2+} Release Channel

Almost all mammalian TRP channels are also located on the intracellular membranes in addition to the plasma membrane [73]. Thus it is not so surprising that, in β-cells TRPM2 is also an intracellular Ca^{2+}-release channel. It is located on the acidic Ca^{2+} stores like the lysosomes [14]. Thus, TRPM2 increases $[Ca^{2+}]_i$ not just by activating the channels located on the plasma membrane but also by release of Ca^{2+} through the TRPM2 channels located on the lysosomal Ca^{2+} stores [14]. Mobilization of Ca^{2+} from the lysosomes is important for externalization of phosphatidylserine, a hallmark of apoptotic cells [74]. It has been speculated that TRPM2 may provide a mechanism for mediating apoptosis and elimination of those β-cells that have been severely damaged by oxidative stress [75].

42.4 TRPM3

TRPM3 has many isoforms, some having different functional properties [76]. TRPM3 channels display some constitutive activity [77, 78], and may thus partly account for the background depolarizing currents in the β-cells. Some sphingolipids e.g. D-*erythro*-sphingosine, dihydro-D-*erythro*-sphingosine and *N,N*-dimethyl-D-*erythro*-sphingosine but not sphingosine-1-phosphate and ceramides activate TRPM3 channel [79]. Interestingly, TRPM3 of β-cells is directly activated by supraphysiological concentrations ($EC5_0 = 23$ μM) of the steroid pregnenolone sulphate (PS), which makes it a useful pharmacological tool for the study of this

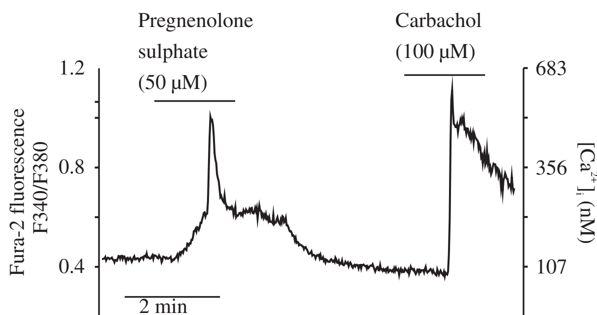

Fig. 42.1 Pregnenolone sulphate, an activator of TRPM3 increases $[Ca^{2+}]_i$ in human β-cells. $[Ca^{2+}]_i$ was measured by microfluometry from fura-2-loaded single human β-cells identified by their size and appearance. Cells plated on coverslips were perfused by modified Kreb's-Ringer bicarbonate buffer containing 3 mM glucose, at 37° C. Pregnenolone sulphate (*PS*) and carbachol were added during the times indicated by the horizontal bars. PS increased $[Ca^{2+}]_i$ which returned to the base line on wash out of the substance. The trace is representative of experiments repeated at least four times

channel [15]. Another low affinity pharmacological activator of TRPM3 is nifedipine ($EC_{50} = \sim 32\ \mu M$), which is commonly used as a potent inhibitor of L-type voltage-gated Ca^{2+} channels.

In in vitro experiments, activation of TRPM3 by high concentration of PS (e.g. 36 μM) increases $[Ca^{2+}]_i$ in mouse islet cells and augments glucose-stimulated insulin secretion. In such experiments, at least 5 μM PS is required to detect TRPM3 channel activity. We have found that PS increases $[Ca^{2+}]_i$ also in human β-cells (Fig. 42.1) suggesting that human β-cells also have functional TRPM3 channels. The concentrations of PS used in these in vitro experiments are too high to be physiologically relevant for regulation of insulin secretion. However, in some conditions e.g. in mid-pregnancy [80], hyperthyroidism [81], anxiety-depressive disorder [82], and 21-hydroxylate deficiency [83], concentration of PS in the plasma is elevated (normal plasma concentration of PS in adult human is ~ 0.2–0.4 μM). It is possible that in such conditions, modest increases in the concentration of PS synergize with other unknown activators of TRPM3 and may thus render the β-cells more excitable.

Another interesting feature of TRPM3 channel is that it conducts Zn^{2+} ions [84]. Zn^{2+} can pass through TRPM3 channels even when concentration of extracellular Zn^{2+} is as low as 10 μM and concentrations of Ca^{2+} and Mg^{2+} are 1–2 mM. Thus TRPM3, like ZnT-8, constitutes one of the pathways for Zn^{2+} transport across the plasma membrane of β-cells [84].

42.5 TRPV4 (Other Names OTRPC4, VR-OAC, VRL-2, TRP-12)

According to the Human Protein Atlas (version 6.0, updated 2010–03–28) published by the Swedish Human Proteome Resource (HPR) program (www.hpr.se), TRPV4

is abundant in the islets, and is almost completely absent in the pancreatic acinar cells. However, reliability of immunohistochemistry depends on control experiments done to elucidate the specificity of the antibody used. One important control experiment that should be employed in IHC is neutralization of the antibody by the corresponding peptide antigen. Rather surprisingly, this control is not included in the protocols used by HPR because it can not be adapted to their high throughput systems. An examination of the TRPV4 antibody developed by HPR (Product number HPA007150, marketed by Atlas Antibodies under category "Prestige Antibodies") shows that it stains mostly in the nuclei and that it does not detect any band in the Western blot (information obtained from HPR website). Thus, IHC results obtained by this antibody are unreliable.

Nevertheless, TRPV4 has been described at least in a mouse insulinoma cell line where it acts as a stretch-activated plasma membrane channel. In MIN6 cells aggregated human islet amyloid polypeptide (hIAPP) interacts with the plasma membrane and increases $[Ca^{2+}]_i$ by activating TRPV4, an observation consistent with the role of TRV4 as a mechanosensitive channel [22]. Increase of $[Ca^{2+}]_i$ by hIAPP and consequent increase in ER stress response are suppressed by siRNA against TRPV4 [22].

TRPV4 can be activated by diverse physical stimuli including warm temperatures (>25–34°C), volume changes [85, 86], osmolality, stretch, and mechanical stimuli [87]. As pointed out by Ilya Digel in Chapter 25 in this book "The difference between mechanosensitive channels and thermosensitive molecules is only the size and the organization of the "exciting" agents – a lot of non-coordinated events (thermal stimuli) versus a net stretch (mechanical stimuli). Therefore, not surprisingly, many members of thermosensing TRPV family are also known as osmo- and mechanosensors". Stimulation of β-cells by glucose generates heat [67] and increases volume of β-cells [88]. It is, thus, possible that such changes may act as physical second messengers to activate the TRPV4 channel of β-cells. TRPV4 may thus be involved in $[Ca^{2+}]_i$ increase in β-cells activated by hypotonicity, volume increase and mechanical stretch [89].

Endogenous chemical agonists of TRPV4 include arachidonic acid, anandamide, and 5,6- or 14,15-epoxyeicosatrienoic acids. Phorbol derivatives 4α-Phorbol 12,13-didecanoate (4α-PDD) and Phorbol 12-myristate 13-acetate activate the channel directly without involving protein kinase C [90]. 4α-PDD is a commonly used pharmacological tool for activation of TRPV4. Other potent agonists for TRPV4 are bisandrographolide A [91] and GSK1016790A [92].

42.6 TRPV2

TRPV2 protein has been demonstrated in mouse insulinoma MIN6 cells and cultured mouse β-cells. In these cells insulin stimulates translocation of TRPV2 from the cytoplasm to the plasma membrane as demonstrated by use of TRPV2 tagged with GFP or c-Myc [21]. Such action of insulin on the TRPV2 protein enhances Ca^{2+} entry, insulin secretion and β-cell growth. These effects are inhibited by

tranilast, a pharmacological inhibitor of TRPV2 or by konock-down of TRPV2 by molecular techniques. Importantly, glucose, by stimulating insulin secretion promotes insulin-induced translocation and insertion of TRPV2 to the plasma membrane, providing a positive feed-back mechanism [21]. It should be noted that TRPV2 current has not yet been demonstrated in any of the insulin secreting cells. Thus, it is not entirely clear whether TRPV2 protein exerts its function by acting as a channel or it acts as a signaling protein independent of its function as an ion channel. It is known that TRPV2 may display some spontaneous basal activity. In β-cells, such constitutive activity of TRPV2 may account partly for the background depolarizing current. Like TRPV4, TRPV2 is also activated by heat, mechanical stretch and osmotic swelling which may possibly couple glucose metabolism to the activation of the channel.

42.7 TRPV1

Rat insulinoma cell lines RIN and INS-1 express TRPV1 [20]. In these cells capsaicin, an activator of TRPV1 increases $[Ca^{2+}]_i$ and the increase can be inhibited by capsazepine. In primary β-cells, capsaicin does not increase $[Ca^{2+}]_i$. TRPV1 immunoreactivity has been described in primary β-cells of Sprague Dawley rats by one group [20], but not in those of Zucker diabetic rats [93] or NOD mice [94]. TRPV1 channels are present in some nerve fibers in mouse islets [94] but we have not observed any TRPV1 positive nerve fiber in human islets.

42.8 TRPC1 and TRPC4

From Human Protein Atlas (version 6.0) it appears that TRPC1 is expressed at high level in the islets and numerous other cells and tissues (www.hpr.se). I find it hard to interpret these data since the antibody (HPA021130, distributed by Atlas Antibodies as one of the “Prestige Antibodies”) does not detect the expected protein band in Western blot (according to the information posted in the HPR website). As mentioned earlier, an important control experiment i.e. the effect of neutralization of the antibody by the corresponding antigen is not included in the protocol used by the Human Proteome Research program. Nevertheless, TRPC1 mRNA has been demonstrated in mouse islets, MIN6 cells, rat β-cells and INS-1 cells [5, 7]. The level of expression of TRPC1 in rat primary β-cells is higher than that in the rat insulinoma INS-1 cells [7]. In MIN6 cells, four splice variants of TRPC1 have been identified. Of these, the β variant is the most abundant one in MIN6 cells and probably also in mouse islets [5]. Different insulinoma cell lines differ in terms of the level of expression of different TRPC channels. Thus, TRPC1 is highly expressed in MIN6 cell and TRPC4 is highly expressed in β-TC3 cells [5, 6]. TRPC4 is also expressed at high level in INS-1 cells and rat β-cells [7]. TRPC4 has at least two major splice variants: the full length TRPC4α and a shorter TRPC4β which lacks 84

amino acids in the C-terminus. In rat β-cells TRPC4β is the main isoform whereas in rat insulinoma INS-1 cells TRPC4α is the main isoform [7]. TRPC5 which is closely related to TRPC4 is not expressed in mouse islets [6].

TRPC1 and TRPC4 are non-selective cation channels with almost equal permeability for Ca^{2+} and Na^{+}. Since these channels are expressed in many cells, it appears less likely that they perform any β-cell-specific function. Nevertheless, these channels are there in the β-cells and one needs to speculate what their roles could be in these cells. It is possible that low level of spontaneous activity of these channels constitutes part of the background depolarizing current in the β-cells. More, importantly, both of these channels are molecular candidates for the non-selective cation current activated by Gq/PI-PLC-coupled receptors or by depletion of the ER Ca^{2+} store. TRPC1 is the most well characterized TRP channel that, according to many investigators, mediates store-operated Ca^{2+} entry (SOCE) in many cells. Such SOCE has been described in electrically excitable cells like β-cells [95], and it is possible that channels like TRPC1 or TRPC4 or their heteromers mediate SOCE in β-cells. However, there are many unresolved issues as to whether the TRPC channels are regulated by the ER Ca^{2+} sensor STIM1 or just by mechanisms dependent on PI-PLC activation [96] (see Chapter 24 by Kwong Tai Cheng, Hwei Ling Ong, Xibao Liu, and Indu S. Ambudkar, in this book). One hypothesis is that hydrolysis of PIP2 by PI-PLC induces shortening of certain membrane lipids and thus alters the lipid packing at the inner surface of the membrane microdomain [see fig 2 of reference [97] for explanation]. Changes in the membrane curvature and in the lipid-TRP channel interaction induce conformational changes and consequent opening of the TRP channels. Such changes in the membrane curvature can also alter the “open channel block” by forcefully removing the metal ions from the channels and this may lead to further opening of the channels [97, 98].

42.9 Perspectives

The presence of at least 8 TRP channels in the β-cells call for a revision of the current views on the mechanisms of insulin secretion in response to various insulin secretagogues. The background depolarizing currents in the β-cells could be mediated by these 8 redundant TRP channels. Consequently knock out of one or other of these channels may not cause any major change in the background depolarizing current and may not lead to readily identifiable phenotypes. When β-cells are in the READY mode, small increases in the inward depolarizing currents mediated through one or other TRP channels may shift the β-cells to the ON mode. The coupling factors between insulin-secretagogues and the activation of the TRP channels could be diverse physical second messengers like heat, swelling, stretch, changes in the curvature of the plasma membrane microdomains, and chemical factors like arachidonic acid, cAMP, PIP_2, Ca^{2+} and other as yet unknown ones. It will be challenging to investigate the quantitative contribution of different second messengers and different TRP channels in β-cells under different physiological and pathological conditions.

Acknowledgments I would like to thank Romain Guinamard and Frank Kühn for useful discussions. I am grateful to Stockholm County Council, Forskningscentrum, Landes Bioscience, and Engelbrechts kliniken. Financial support was obtained from the Swedish Research Council

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