Signal transduction via TRPM3 channels in pancreatic β -cells

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Abstract

Transient receptor potential melastatin 3 (TRPM3) channels are non-selective cation channels that are expressed in insulinoma cells and pancreatic β -cells. Stimulation of TRPM3 with the neurosteroid pregnenolone sulfate induces an intracellular signaling cascade, involving a rise in intracellular Ca^{2+} concentration, activation of the protein kinases Raf and ERK, and a change in the gene expression pattern of the cells. In particular, biosynthesis of insulin is altered following activation of TRPM3 by pregnenolone sulfate. Moreover, a direct effect of TRPM3 stimulation on insulin secretion has been reported. The fact that stimulation of TRPM3 induces a signaling cascade that is very similar to the signaling cascade induced by glucose in β -cells suggests that TRPM3 may influence main functions of pancreatic β -cells. The view that TRPM3 represents an ionotropic steroid receptor of pancreatic β -cells linking insulin release with steroid hormone signaling is discussed.

Key Words

- ▶ Gene regulation
- ▶ Pancreatic β -cell
- ▶ Signal transduction
- ▶ Steroids
- ▶ Calcium signaling

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Introduction

Transient receptor potential (TRP) channels are a heterogeneous superfamily of cation influx channels, separated in the subfamilies TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPML (mucolipins), TRPP (polycystin), and TRPA (ankyrin). Many biological functions have been connected with TRP channel activation, including regulation of Ca^{2+} homeostasis, tumorigenesis, sensory temperature, and pain sensation.

In pancreatic β -cells, several members of the TRP family of cation channel proteins are expressed (Colsoul *et al.* 2011, Islam 2011). The channels with the highest expression are TRPML1 and TRPP2. Functional roles have been attributed to TRPA1, TRPM2, and TRPM5. Activation of TRPA1 channels by the agonist allyl isothiocyanate stimulates insulin release from insulinoma cells and primary isolated pancreatic β -cells (Cao *et al.* 2012). Secretion was inhibited following incubation of the cells with a TRPA1-specific antagonist, confirming the connection between TRPA1 channel activation and insulin

secretion. TRPM2 is activated by H_2O_2 and reactive oxygen species. Accordingly, TRPM2 function has been correlated with β -cell apoptosis (Hara *et al.* 2002). TRPM2-deficient mice have higher basal glucose levels and an impaired glucose tolerance (Uchida *et al.* 2011). TRPM5 channels are important constituents of Ca^{2+} -activated cation current in β -cells. Correspondingly, reduced channel activity could be measured in β -cells of TRPM5-deficient mice (Colsoul *et al.* 2010). Moreover, TRPM5 is involved in glucose-induced insulin release as demonstrated by impaired glucose tolerance performed with TRPM5-deficient mice, due to reduced plasma insulin levels (Brixel *et al.* 2010, Colsoul *et al.* 2010).

TRPM3 channels

TRPM3 has the typical features of TRP channels, including six transmembrane domains and a pore domain between the fifth and sixth transmembrane domain. Both amino

and carboxy termini are located in the cytosol. TRPM3 contains the TRP domain, a highly conserved motif, located on the C-terminal side of the sixth transmembrane domain. In addition, calmodulin-binding sites have been detected within the N-terminal cytosolic domain of TRPM3 (Halokovska *et al.* 2012). The modular structure of TRPM3 is depicted in Fig. 1A.

The TRPM3 gene encodes many TRPM3 channel isoforms due to alternative splicing (Lee *et al.* 2003,

Figure 1

Modular structure, membrane topology, and expression of TRPM3. (A) Schematic representation of the modular structure of TRPM3 channels. TRPM3 has six transmembrane domains with a pore-forming domain between transmembrane regions 5 and 6. Both the N- and C-termini are located in the cytosol. The N-terminus contains two calmodulin-binding sites encompassing amino acids 35–124 and 291–382. The C-terminus contains the TRP domain on the C-terminal side of the sixth transmembrane domain. (B) Primary sequence of the putative pore region of the TRPM3 splice variants TRPM3 α 1 and TRPM3 α 2 (Oberwinkler *et al.* 2005). (C) Expression of TRPM3 and voltage-gated Ca^{2+} channels Cav1.2 and Cav1.3 in islets and insulinoma cells. RNA was prepared from islets and INS1 insulinoma cells. Expression of TRPM3 was monitored via RT-PCR using gene-specific primers. As a control, expression of GAPDH was assessed (reproduced from Müller I, Rössler OG & Thiel G 2011 Pregnenolone sulfate activates basic region leucine zipper transcription factors in insulinoma cells: role of voltage-gated Ca^{2+} channels and transient receptor potential melastatin 3 channels. *Molecular Pharmacology* 80 1179–1189 with kind permission from the American Society for Pharmacology and Experimental Therapeutics).

Grimm *et al.* 2005, Oberwinkler *et al.* 2005). In fact, TRPM3 encompasses the largest number of splice variants within the TRP family of channel proteins. Of particular interest are the TRPM3 isoforms, TRPM3 α 1 and TRPM3 α 2, that differ within the pore region of the channel between the fifth and sixth transmembrane region (Fig. 1B). Within this pore region, TRPM3 α 1 has 12 additional amino acids in comparison to TRPM3 α 2. In addition, TRPM3 α 1 has an alanine residue at the C-terminal of the insertion, while TRPM3 α 2 has a proline residue at this position. Physiological experiments revealed that TRPM3 α 1 channels are poorly permeable for divalent cations, while TRPM3 α 2 channels are at least 10 times more permeable for Ca^{2+} and at least 100 times more permeable for Mg^{2+} (Oberwinkler *et al.* 2005). Thus, alternative splicing produces TRPM3 channels of entirely different cation permeability, by changing the primary sequence of the pore region of the channel. In addition, a TRPM3 splice variant termed TRPM3 α 7 that lacks amino acid residues 512–529 displays no ionic current (Frühwald *et al.* 2012), suggesting that this variant functions as a decoy receptor. In INS1 insulinoma cells, this variant makes up 7.2% of the TRPM3 transcripts. TRPM3 α 7 forms heteromeric complexes with TRPM3 α 2, leading to a reduced Ca^{2+} influx following stimulation (Frühwald *et al.* 2012).

TRPM3 channels are prominently expressed in various tissues, including kidney, liver, ovary, brain, spinal cord, pituitary, vascular smooth muscle, and testis (Grimm *et al.* 2003, Lee *et al.* 2003, Naylor *et al.* 2010). Interestingly, TRPM3 is expressed in insulinoma cells and β -cells of the pancreas, as shown in Fig. 1C.

Ligands and inhibitors of TRPM3 channels

TRPM3 channels exhibit constitutive activity following overexpression in HEK293 cells (Grimm *et al.* 2003, 2005, Oberwinkler *et al.* 2005). Low-level constitutive TRPM3 activity has also been detected in vascular smooth muscle cells (Naylor *et al.* 2010).

TRPM3 is activated by β -cyclodextrin, while the role of D-erythro-sphingosine as a TRPM3 ligand is a matter of debate and may depend on the experimental design (Grimm *et al.* 2005, Wagner *et al.* 2008, Naylor *et al.* 2010). Surprisingly, TRPM3 channels are activated by the dihydropyridine nifedipine, an inhibitor of voltage-gated Ca^{2+} channels, while the structurally related compounds nimodipine, nifedipine, and nitrendipine were inactive (Wagner *et al.* 2008). Cholesterol, the precursor metabolite of pregnenolone, progesterone, and mefenamic acid prevents TRPM3 channel activation (Naylor *et al.* 2010,

Klose *et al.* 2011, Majeed *et al.* 2012). The antidiabetic PPAR γ -agonists rosiglitazone and troglitazone function as TRPM3 channel blockers (Majeed *et al.* 2011). Recently, the screening of a compound library revealed that citrus fruit flavanones such as naringenin and hesperetin and fabacea secondary metabolites selectively inhibit TRPM3 channel activation (Straub *et al.* 2013).

The neurosteroid pregnenolone sulfate, the sulfated form of pregnenolone, has been found to activate TRPM3 channels in insulinoma cells and pancreatic β -cells (Wagner *et al.* 2008, Klose *et al.* 2011, Mayer *et al.* 2011, Müller *et al.* 2011) as well as in other cell types. Stimulation with pregnenolone sulfate also activates TRPM3 channels in HEK293 cells, vascular smooth muscle cells, and synovial fibroblasts (Ciurtin *et al.* 2010, Majeed *et al.* 2010, Naylor *et al.* 2010, Klose *et al.* 2011), confirming that TRPM3 functions as a Ca^{2+} -permeable non-selective cation channel. Furthermore, studies involving TRPM3-deficient mice revealed that TRPM3 is the major receptor for pregnenolone sulfate in dorsal root and trigeminal ganglia (Vriens *et al.* 2011). The fact that pregnenolone sulfate stimulates TRPM3 channel activity is the basis for the hypothesis that TRPM3 functions as an ionotropic steroid receptor of pancreatic β -cells linking insulin release with steroid hormone signaling (Frühwald *et al.* 2012).

Pregnenolone sulfate: a ligand for different channels and receptors

Pregnenolone sulfate is not a TRPM3-specific ligand, as this steroid also exerts modulatory effects on several types of receptors and ion channels including the *N*-methyl-D-aspartate (NMDA) receptor, the gamma amino butyric acid-A receptor, voltage-gated Ca^{2+} channels, and Kir2.3 K^+ channels (Horak *et al.* 2004, Chen & Sokabe 2005, Hige *et al.* 2006, Kobayashi *et al.* 2009, Chen *et al.* 2010, Kostakis *et al.* 2011, Mayer *et al.* 2011, Wang 2011). The analysis of chimeric NMDA receptors composed of NR2B and NR2D domains demonstrated that a 'steroid modulatory domain' (SMD1) on the NMDA receptor subunit NR2B is crucial for the stimulation of NR2B-containing NMDA receptors by pregnenolone sulfate. This domain encompasses the extracellular located J/K helices of the receptor as well as the contiguous fourth trans-membrane domain (Jang *et al.* 2004). The identification of SMD1 provides a structural component for the pregnenolone sulfate-induced activation of NR2B-containing NMDA receptors.

The site attributed to pregnenolone sulfate binding to TRPM3 channels has yet to be identified. Overlay

experiments revealed that pregnenolone sulfate, progesterone, and dihydrotestosterone bound to TRPM3 *in vitro* (Majeed *et al.* 2012). Given the fact that other members of the TRPM subfamily (TRPM2, TRPM7, TRPM8) are not activated by pregnenolone sulfate (Wagner *et al.* 2008), the strategy to generate and analyze chimeric TRP channels composed of TRPM3 and one of the other pregnenolone sulfate-insensitive TRPM channels may lead to the identification of a 'SMD1' within the TRPM3 molecule. The chemical requirements for steroids to activate TRPM3 channels have been investigated using HEK293 cells expressing TRPM3 channels. The results revealed the importance of a sulfate group at ring A and a *cis* (β) configuration of the side group of pregnenolone sulfate (Majeed *et al.* 2010). Steroids including progesterone, 17 β -estradiol, 17 β -estradiol sulfate, dihydrotestosterone, aldosterone, cortisol, and vitamins D₂ and D₃ were ineffective in the stimulation of Ca^{2+} -influx via the TRPM3 channel (Majeed *et al.* 2010). These results argue for the existence of a specific and unique steroid binding site on the TRPM3 molecule.

Regulation of intracellular Ca^{2+} concentration in pancreatic β -cells by TRPM3 and voltage-gated Ca^{2+} channels

In pancreatic β -cells, the closure of K_{ATP} -regulated potassium channels following glucose uptake and metabolism induces the depolarization of the plasma membrane due to the activation of L-type voltage-gated Ca^{2+} channels. Ca^{2+} influx via these voltage-dependent Ca^{2+} channels is necessary for glucose-induced insulin secretion. Accordingly, pharmacological inhibition of voltage-gated Ca^{2+} channels blocks glucose-induced insulin secretion as well as glucose-induced transcription (Trus *et al.* 2007, Mayer & Thiel 2009, Yang *et al.* 2010). Likewise, pharmacological activation of voltage-gated Ca^{2+} channels increases insulin secretion in the absence of glucose (Yang *et al.* 2010). Insulin-secreting INS1 insulinoma cells and pancreatic β -cells are responsive to pregnenolone sulfate stimulation, leading to a rapid influx of Ca^{2+} ions into the cells (Wagner *et al.* 2008, Islam 2011; Fig. 2). This effect is based on the expression of Ca^{2+} -permeable splice variants such as TRPM3 α 2 in pancreatic β -cells. Intracellular signaling induced by both activation of L-type Ca^{2+} channels or TRPM3 channels is impaired by chelating extracellular or intracellular $[\text{Ca}^{2+}]$ (Mayer *et al.* 2011). Experiments involving expression of a TRPM3-specific short hairpin RNA revealed that TRPM3 is required to induce the Ca^{2+} -dependent

Figure 2

Ca^{2+} influx into human pancreatic β -cells following stimulation with pregnenolone sulfate ($50 \mu\text{M}$). As a control, cells were stimulated with carbachol ($100 \mu\text{M}$). $[\text{Ca}^{2+}]_i$ was measured from fura-2-loaded β -cells (reproduced with modifications from Islam MS 2011 TRP channels of islets. In: Transient receptor potential channels. *Advances in Experimental Medicine and Biology* 704 811–830 (Fig. 42.1) with kind permission from Springer Science+Business Media B.V.).

intracellular signaling cascade in pregnenolone sulfate-stimulated insulinoma cells that are maintained in the medium containing low glucose concentrations (2 mM). However, the initial Ca^{2+} influx into the cells, mediated by TRPM3 activation, is not sufficient to induce an intracellular signaling cascade, leading to changes in gene transcription. Rather, activation of voltage-gated Ca^{2+} channels is additionally required, as incubation of the cells with verapamil, a voltage-gated Ca^{2+} channel blocker, inhibits the signaling cascade induced by pregnenolone sulfate under these conditions (Mayer *et al.* 2011; Fig. 3). Thus, we propose that stimulation of TRPM3 with pregnenolone sulfate induces a depolarization of the plasma membrane, leading to the activation of L-type voltage-gated Ca^{2+} channels, a further influx of Ca^{2+} into the cells, and the initiation of a signaling cascade that triggers changes in the gene expression pattern of the cells.

Using either intracellular Ca^{2+} measurement with Ca^{2+} indicators and/or whole-cell patch-clamp to measure cationic membrane current, TRPM3 stimulation by pregnenolone sulfate is independent of L-type voltage-gated Ca^{2+} channels. This has been proven in heterologous expression studies of TRPM3 in HEK293 cells that are devoid of L-type voltage-gated Ca^{2+} channels (Wagner *et al.* 2008, Majeed *et al.* 2010). Thus, in a heterologous system, TRPM3 functions as a ligand-activated ionotropic receptor in the absence of L-type voltage-gated Ca^{2+} channels, leading to an influx of Ca^{2+} into the cells following stimulation with pregnenolone sulfate. It is not yet known whether the influx of Ca^{2+} through TRPM3 channels is sufficient to induce an

intracellular signaling cascade in HEK293 cells that triggers changes of the gene expression program of the cells.

Experiments performed with pituitary corticotrophs that express functional L-type Ca^{2+} channels, but only trace amounts of TRPM3 revealed that pregnenolone sulfate stimulation does not induce an intracellular signaling cascade (Müller *et al.* 2011), indicating that L-type Ca^{2+} channels do not function as pregnenolone sulfate receptors in this cell type. By contrast, stimulation of insulinoma cells, which are maintained in the medium containing 11 mM glucose, with pregnenolone sulfate induces an intracellular signaling cascade that requires L-type voltage-gated Ca^{2+} channels and is independent of TRPM3 activation (Mayer *et al.* 2011). It is not clear whether pregnenolone sulfate directly binds to L-type Ca^{2+} channels.

Protein kinases and protein phosphatases involved in TRPM3 signaling in pancreatic β -cells

Elevation of $[\text{Ca}^{2+}]_i$ triggers an activation of the protein kinases Raf and ERK in pancreatic β -cells and insulinoma cells (Benes *et al.* 1998, 1999, Arnette *et al.* 2003, Mayer & Thiel 2009, Duan & Cobb 2010). The connection between an elevated Ca^{2+} concentration and the activation of the ERK signaling pathway is accomplished by protein kinase C (PKC), most likely $\text{PKC}\alpha$ and $\text{PKC}\beta\text{II}$ (Benes *et al.* 1999) and the protein kinase Raf (Kolch *et al.* 1993, Corbit *et al.* 2003, Duan & Cobb 2010). Given the similarities between glucose and pregnenolone sulfate-induced signaling, it is not surprising that Raf and ERK are activated in pregnenolone sulfate-stimulated insulinoma cells (Mayer *et al.* 2011). Moreover, pregnenolone sulfate-induced signaling is impaired in insulinoma cells expressing MKP1, a nuclear protein phosphatase that dephosphorylates and inactivates ERK in the nucleus. Thus MKP1 is part of a negative feedback loop inducing dephosphorylation and inactivation of nuclear ERK. In addition, elevated $[\text{Ca}^{2+}]_i$ may activate calcineurin, a Ca^{2+} -regulated protein phosphatase. Calcineurin is composed of two polypeptides, calcineurin A and B. Expression of a constitutively active calcineurin A mutant, which lacks the calmodulin-binding site and the C-terminal autoinhibitory domain and which does not require Ca^{2+} ions for activation, impairs pregnenolone sulfate-regulated signaling in the nucleus (Mayer *et al.* 2011), suggesting that calcineurin may be part of a second negative feedback loop in the pancreatic β -cells (Fig. 3).

Figure 3

Signal transduction of pregnenolone sulfate in pancreatic β -cells. Stimulation of insulinoma cells cultured in medium containing 2 mM glucose with pregnenolone sulfate requires both TRPM3 and L-type voltage-gated Ca^{2+} channels to induce an intracellular signaling cascade. The cytosolic Ca^{2+} concentration is increased via an influx of Ca^{2+} ions from outside. Elevation of the intracellular Ca^{2+} concentration leads to the activation of the ERK signaling cascade, mediated by PKC. A major nuclear substrate for ERK is the ternary complex factor Elk1, an essential component of the

serum response element (SRE) ternary complex. The Egr1 promoter contains five SREs that mediate signal-induced activation of Egr1 gene transcription. Activated ERK phosphorylates in the nucleus the protein kinase MSK that in turn phosphorylates and activates the transcription factor CREB. The phosphatases MKP1 and calcineurin (CaN) function as negative regulators of this signaling cascade by dephosphorylating ERK and Elk1 respectively.

Activation of gene transcription in insulinoma cells and pancreatic β -cells following activation of TRPM3 channels

Elevated levels of glucose have a profound effect on gene transcription in pancreatic β -cells. Several transcription factors are activated in glucose-stimulated insulinoma or pancreatic β -cells, including Egr1 (Frödin *et al.* 1995, Josefsen *et al.* 1999, Bernal-Mizrachi *et al.* 2000, Mayer & Thiel 2009), CREB (Wang *et al.* 2008, Mayer & Thiel 2009), Elk1 (Bernal-Mizrachi *et al.* 2001, Mayer & Thiel 2009), c-Jun, and c-Fos (Glauser & Schlegel 2007, Müller *et al.* 2012a). Stimulation of insulinoma cells with pregnenolone sulfate activates the biosynthesis of Egr1, a Ca^{2+} -regulated zinc finger transcription factor (Thiel *et al.*

2010), while incubation of the cells with pregnenolone or progesterone has no effect on the Egr1 level within the cells (Mayer *et al.* 2011).

The activity of the transcription factor activator protein 1 (AP1) is prominently upregulated in glucose-stimulated insulinoma cells (Glauser *et al.* 2006, Müller *et al.* 2010, 2012a). Pregnenolone sulfate stimulation also leads to a significant elevation of AP1 activity in insulinoma cells (Müller *et al.* 2011). The AP1 transcription factor was originally described as a heterodimer of the transcription factors c-Jun and c-Fos. These proteins dimerize via their leucine zipper domains, which in turn bring together their basic domains to bind to DNA in a sequence-specific manner. Stimulation with pregnenolone sulfate increases the expression of c-Fos and c-Jun.

c-Jun is a substrate for c-Jun N-terminal protein kinases and phosphorylation of c-Jun is required for the activation of the transcriptional activation potential of c-Jun. Accordingly, stimulation of insulinoma cells with pregnenolone sulfate induces the phosphorylation of c-Jun, indicating that it was activated as a result of stimulation (Müller *et al.* 2011). In addition, phosphorylation of CREB is induced and transcription of a cAMP response element (CRE)-controlled reporter gene is stimulated following pregnenolone sulfate treatment (Fig. 3). In each case, the requirement of TRPM3 has been demonstrated by expressing TRPM3-specific short hairpin RNAs (Mayer *et al.* 2011, Müller *et al.* 2011). Hence, pregnenolone sulfate stimulation activates a plethora of transcriptional events in insulinoma cells and pancreatic β -cells, suggesting that transcriptional changes may be important for the biological functions of TRPM3 stimulation (Fig. 3).

Regulation of insulin biosynthesis and secretion by TRPM3

The activation of transcription factors in pregnenolone sulfate-stimulated insulinoma cells and pancreatic β -cells requires the identification of pregnenolone sulfate-regulated genes in these cells and the correlation of the biological functions of these gene products with important physiological parameters in the endocrine pancreas. We recently showed that Egr transcription factors play an important role in controlling insulin biosynthesis, glucose homeostasis, and proliferation/cell death of pancreatic β -cells *in vivo* (Müller *et al.* 2012b). Interestingly, the glucose-responsive transcription factor Egr1 induces insulin gene transcription *in vitro* via activation of the transcription factor pancreas duodenum homeobox 1 (PDX1) in insulinoma cells (Eto *et al.* 2006, 2007), providing a link between glucose sensing and transcription of the insulin gene. PDX1 is a major regulator of insulin expression in pancreatic β -cells (Andrali *et al.* 2008). The regulation of PDX1 expression by Egr transcription factors was confirmed *in vivo* in the analysis of a transgenic mouse model expressing a dominant-negative mutant of Egr1 (Müller *et al.* 2012b). Accordingly, it is conceivable that pregnenolone sulfate-induced stimulation of TRPM3 activates PDX1 expression as well. In fact, Egr1 binds to the PDX1 gene under physiological conditions. Given the fact that PDX1 regulates insulin expression, pregnenolone sulfate stimulation enhances transcription of an insulin promoter/luciferase reporter gene and elevated insulin mRNA levels are detectable

in pregnenolone sulfate-stimulated insulinoma cells (Mayer *et al.* 2011).

A direct effect of pregnenolone sulfate stimulation upon insulin secretion of insulinoma cells has been shown at glucose concentrations >6 mM. However, insulin release from pancreatic islets cultured at low glucose concentration (3 mM) increased only insignificantly, although pregnenolone sulfate stimulation elicited strong Ca^{2+} signals under these conditions (Wagner *et al.* 2008). This study did not distinguish between either TRPM3 and/or L-type voltage-gated Ca^{2+} channel activation in pregnenolone sulfate-induced insulin secretion. Recently, an additive effect of tolbutamide and pregnenolone sulfate stimulation on insulin secretion of insulinoma cells has been reported, indicating that pregnenolone sulfate-induced stimulation of TRPM3 contributes to the amount of secreted insulin. The fact that mefenamic acid reduced pregnenolone sulfate-induced insulin secretion supports the view that activation of TRPM3 channels are involved in the regulation of insulin secretion (Klose *et al.* 2011). TRPM3 also regulates exocytosis in other cellular systems, including interleukin secretion from vascular smooth muscle cells, hyaluronan secretion from synovial fibroblasts, and neurotransmitter release (Zheng 2009, Ciurtin *et al.* 2010, Naylor *et al.* 2010). Currently, the molecular mechanism of how TRPM3 stimulation influences the exocytotic machinery in pancreatic β -cells is unknown. Given the fact that TRPM3 regulates PDX1 expression via controlling Egr1 biosynthesis, it is possible that elevated PDX1 levels are – at least in part – responsible for this effect. This hypothesis is based on the observation that transgenic mice with an inactivated PDX1 allele secrete less insulin (Brissova *et al.* 2002). PDX1 also directly influences the exocytotic machinery by regulating expression of synaptotagmin 1 (Nakajima-Nagata *et al.* 2004), a Ca^{2+} sensor involved in Ca^{2+} -dependent insulin secretion.

TRPM3: an ionotropic steroid receptor of pancreatic β -cells

The discovery that pregnenolone sulfate activates TRPM3 channels has boosted the research concerning the function of TRPM3. However, the concentration of pregnenolone sulfate required to stimulate TRPM3 channels is in the micromolar range, suggesting that pregnenolone sulfate is not a physiological agonist of TRPM3 and may have only pharmacological relevance. Typical pregnenolone sulfate concentrations measured in plasma are rather in the nanomolar and not in the micromolar range. Accordingly, it has been questioned that pregnenolone sulfate plays

an important physiological role in insulin secretion (Colsoul *et al.* 2011). However, we would like to emphasize that human TRPM3 channels may have a greater sensitivity toward pregnenolone sulfate, the murine counterpart, as observed in Ca^{2+} measurement assays (Majeed *et al.* 2010).

The hypothesis that TRPM3 functions as an ionotropic steroid receptor in pancreatic β -cells responsible for modulating insulin biosynthesis and secretion implies that pregnenolone sulfate is synthesized and released in a stimulus-dependent manner. In particular, pregnenolone sulfate concentrations should be regulated by the metabolic state of the organism in order to trigger insulin secretion when insulin is required. The biosynthesis of pregnenolone sulfate starts from cholesterol that is converted to pregnenolone catalyzed by the cytochrome P450 side-chain cleavage enzyme (P450 scc). The sulfonation of pregnenolone to pregnenolone sulfate is catalyzed by hydroxysteroid sulfotransferases and involves the transfer of a sulfonate group from 3'-phosphoadenosine 5'-phosphosulfate to the substrate. The family of cytosolic sulfotransferases includes the enzymes SULT2A1 and SULT2B1. Differential splicing of the SULT2B1 gene generates two isoforms, SULT2B1a and SULT2B1b (Shimizu *et al.* 2003), where SULT2B1a actively sulfonates pregnenolone and thus functions as a pregnenolone sulfotransferase. There are no data available that support the view that pregnenolone sulfate is specifically synthesized and released under conditions where insulin biosynthesis and secretion is required. The fact that TRPM-deficient mice did not show alterations in resting blood glucose levels (Vriens *et al.* 2011) indicates that TRPM3 plays no or only a marginal role in controlling β -cell functions – in contrast to the TRPM2 and TRPM5-deficient mice that showed a pre-diabetic phenotype. Nevertheless, the availability of a TRPM3-deficient transgenic mouse model, together with the recently identified pharmacological agonists and antagonists of TRPM3 (Wagner *et al.* 2008, Klose *et al.* 2011), will certainly support the functional analysis of TRPM3 channel proteins in the future.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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