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TRPM3 in temperature sensing and beyond

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Abbreviations: TRP, Transient receptor potential; TRPV, Transient receptor potential vanilloid; TRPM, Transient receptor potential melastatin; DRG, Dorsal root ganglion; TG, Trigeminal ganglion; PCR, Polymerase chain reaction; PS, Pregnenolone sulfate; Clt, Clotrimazole; Q_{10}, 10-degree temperature coefficient; DeSPH, D-erythro-sphingosine; RT, Room temperature; DHEA, Dehydroepiandrosterone; PPAR-γ, Peroxisome proliferator-activator receptor - γ; ΔH, Enthalpy; ΔS, Entropy; ΔG, Gibbs free energy.

TRPM3, also known as melastatin 2 (MLSN2), LTRPC3 (long TRPC3) and KIAA1616, is a member of the TRPM subfamily of transient receptor potential (TRP) ion channels. The channel was originally identified as a volume-regulated ion channel that can be activated upon reduction of the extracellular osmolality. Later, the channel was proposed to be involved in the modulation of insulin release in pancreatic islets. However, new evidence has uncovered a role of TRPM3 as a thermosensitive nociceptor channel implicated in the detection of noxious heat. The channel is functionally expressed in a subset of neurons of the somatosensory system and can be activated by heat. The purpose of the present review is to summarize existing knowledge of the expression, biophysics and pharmacology of TRPM3 and to serve as a guide for future studies aimed at improving the understanding of the mechanism of thermosensation and proposed physiological functions of TRPM3.

Introduction

Thermal cues from skin and mouth stimuli are conveyed by primary afferent sensory neurons that have their cell bodies in the trigeminal and dorsal root ganglia. Temperature-sensitive cation channels of the transient receptor potential (TRP) superfamily are highly expressed in the somatosensory system and their activation characteristics cover the entire range of temperatures that mammals can discriminate.[1] TRPV1, the channel that can be activated by capsaicin, the hot ingredient of capsicum peppers, was identified as the first temperature sensitive TRP channel.[2] TRPV1 is expressed in a subset of nociceptive, small diameter Aδ and C fibers originating from dorsal root ganglion (DRG) and trigeminal ganglion (TG) neurons. In support of its role as a sensor of thermal and chemical stimuli, currents evoked by heat (>43°C) and capsaicin were virtually absent in cultured neurons from TRPV1 knockout mice, whereas higher threshold (>55°C) heat responses were intact.[3,4] Although TRPV1 has been proposed to be as the principal heat responsive channel for nociceptive neurons, no significant difference between TRPV1 deficient mice and their wild type littermates was observed using single-fiber electrical recordings of primary afferent C-fibers.[5–7] The residual heat sensitivity in TRPV1-deficient mice suggested the existence of one or more additional noxious heat sensor(s), and 3 closely related members of the TRPV subfamily, TRPV2-TRPV4, were obvious candidates.[8–13] However, a recent report demonstrated that genetic ablation of TRPV2 in mice has no discernible effect on various aspects of thermo sensing in mice, even when TRPV1 activity is simultaneously suppressed.[14] Moreover, the human TRPV2 ortholog appears to be insensitive to warmth.[15] TRPV3 and TRPV4 have been put forward as molecular sensor involved in the detection of innocuous warmth.[9–12] But recent studies indicate that the alteration in temperature preference in TRPV3-deficient mice is highly dependent on the genetic background and gender of the mice.[16,17] Therefore, TRPV2-TRPV4 can be excluded as key thermosensors in the somatosensory system. Recently, the transient receptor potential melastatin-3 (TRPM3) was identified as an alternative noxious heat sensor.[18] This review will summarize and discuss the present knowledge of the *Trpm3* gene product, and will address the mechanism of thermosensation and proposed functions of TRPM3 in temperature sensing and beyond.

Expression of TRPM3

The *trpm3* gene is localized on chromosome 9q21.12 in human and on chromosome 19qb in mouse.[19,20] The complete

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TRPM3 gene consists of 28 exons and possesses several alternative splice sites,[21-23] which results in a tremendous amount (up to 20) of isoforms[20,21] (for detailed review see[24]). Until today, no fingerprint exists of all different TRPM3 isoforms in cellular tissues and therefore the physiological relevance and function of those isoforms stays unexplored. The difficulties to obtain a distribution map are caused by the high isoform similarities that pose a challenge to produce specific primers and antibodies. In this review, we will focus on the functional properties of the TRPM3α2 isoform (hereafter named TRPM3), which are also observed in the human TRPM3 isoform and the endogenously expressed TRPM3 isoform in mouse. However, the role of other TRPM3 isoforms cannot be excluded in specific tissues or (patho)physiological conditions.

Like most of the TRP channels, TRPM3 is expressed in a wide variety of tissues. High expression of TRPM3 is found in the brain, spinal cord, sensory neurons, pituitary, kidney, eye, testis and adipose tissue.[19,25-29] In addition, it is expressed in sperm cells, ovaries, pancreas, heart, blood vessels and bladder.[22,27,30-33] A detailed overview of TRPM3 expressing tissues and cell types is given by Oberwinkler et al.[24]

Pain and temperature responses are mediated by medium diameter myelinated Aδ (fast pain) and small diameter unmyelinated C fibers (slow pain).[1,34] Using quantitative real time-PCR and *in situ* hybridization TRPM3 mRNA was detected in mouse DRG and TG, at levels comparable to that of TRPV1.[18,36] Western blot analysis demonstrated TRPM3 protein expression in DRG and TG tissue from *Trpm3*$^{+/+}$ but not from *Trpm3*$^{-/-}$ mice. Functional TRPM3 in the somatosensory system is, like TRPV1, restricted almost exclusively to small-diameter (<25 μm) DRG neurons, which are known to include unmyelinated nociceptor neurons.[18] Altogether, this knowledge showed evidence for the functional expression of TRPM3 in the somatosensory system.

Functional Properties

TRPM3 functions as a Ca^{2+}-permeable, non-selective cation channel (P_{Ca}^{2+}/P_{Cs+} of 10) and is inhibited by intracellular Mg^{2+} [20, 37]. TRPM3 channels are nearly equally permeable to different monovalent anorganic cations and show 4–10 times higher relative permeabilities for divalent cations.[37] Like all TRP channels, TRPM3 contains 6 transmembrane spanning regions with a pore-forming reentrant loop between the fifth (S5) and the sixth (S6) transmembrane domain. Both the amino (N)- and carboxyl (C)- termini are located intracellularly.[38,39] At this point, only smaller parts of a limited number of TRP proteins have been crystallized, whereas the structure of TRPV1 has recently been determined by electron cryo-microscopy. Given the restricted homology between TRPM3 and channels or channel fragments whose structures have been determined, our knowledge of the 3-dimensional structure of the channel remains limited.[38,39] Functional TRP channels are tetramers[40,41] and in addition to homotetrameric TRPM3 channels, there are an increasing number of examples of heteromultimeric TRPM3 channel assembly, both between different TRPM3 isoforms[21] and with the closely related TRPM1 channel.[42] Regrettably, the heteromultimeric TRPM3 channels are always described in an overexpression system, and no evidence is available yet for a heteromultimeric TRPM3 channel in a native tissue.

It was generally accepted that in TRP channels physical and chemical activating stimuli lead to gating of a single, central cation-conducting pore formed by S5, S6 and the interconnecting pore loop.[43] For TRPM3, stimulation by heat or chemical compounds like pregnenolone sulfate (PS) and nifedipine, opens the central pore and induces outwardly rectifying currents in TRPM3-expressing cells (**Fig. 1**). The central pore is highly permeable for Ca^{2+} and Mg^{2+} and can be blocked by the nonspecific TRP channel blocker La^{3+} [20, 44]. At −150 mV, single-channel currents were measured with an average amplitude of −7.1 pA and an estimated single-channel conductance of 50 pS.[44] Interestingly, recent evidence indicates the existence of an alternative ion permeation pathway in TRPM3, distinct form the central pore, that can be specifically activated by the combined application of PS and clotrimazole (Clt), a drug used to treat yeast infections.[44] Remarkably, opening of the alternative permeation pathway is stimulus-dependent, since co-application of Clt with heat or nifedipine did not induce the opening of the alternative pathway (**Fig. 2**).[44] This stimulus dependency may indicate that the channel has to be in a specific conformation to induce opening

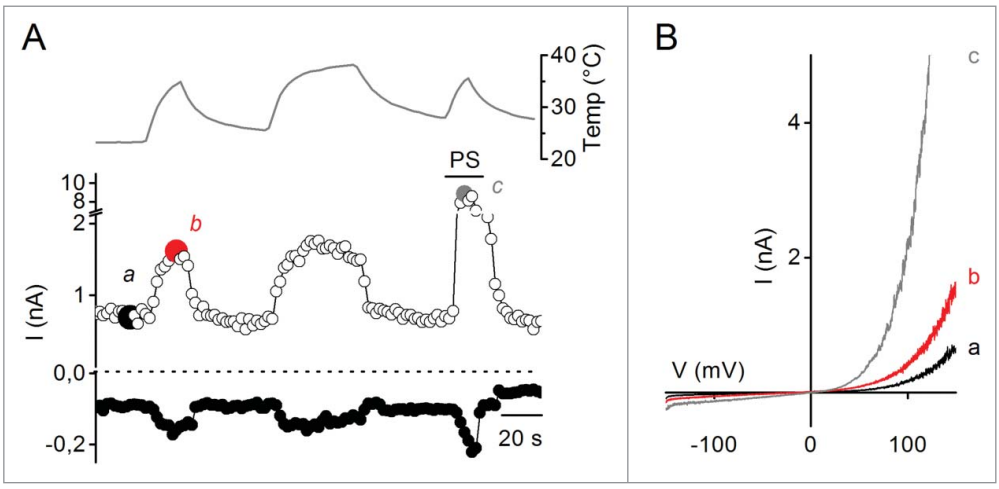

Figure 1. TRPM3 channel is activated by heat. (**A**) Time course of whole-cell TRPM3 expressing HEK293 cell currents at ±80 mV during heating. At the indicated time point 40 μM pregnenolone sulfate (PS) is added. (**B**) Current (I) -Voltage (V) relations were obtained at the indicated time points in panel (**A**). Figure adapted from Vriens J et al. 2011 Neuron May 12;70(3):482–94. Doi: 10.1016/j.neuron.2011.02.051

of the alternative pore by Clt. This hypothesis is supported by the fact that the putative PS-interacting domain is located at the extracellular site of TRPM3, which differs from the nifedipine-interacting domain.[44–47] Typically, this alternative ion permeation pathway shows (*i*) a strong inward rectification, (*ii*) a low permeability to Ca^{2+} and Mg^{2+}, (*iii*) a single-channel conductance of ~20 pS (*iv*) resistance to Ca^{2+}-dependent desensitization and (*v*) a low sensitivity by the open pore blocker La^{3+} (**Fig. 3**).[44] The presence of an alternative ion permeation pathway is also demonstrated in voltage-gated Na^+ and K^+ cation channels, where current leaking through the voltage sensor domains (S4) are termed 'omega current' or 'gating pore current'.[48,49] These gating pores are unveiled by mutations of the highly conserved positively charged amino acids in the S4 segment, which disrupt interactions between the S4 segment and the gating charge transfer center.[50] There is increasing evidence that gating pores in voltage-gated channels are linked to several familial diseases, including mixed peripheral nerve hyperexcitability.[51–53] In contrast to the alternative pathway in classical voltage-gated cation channels, which is induced by artificial or disease-related mutations, the inwardly rectifying TRPM3 current component exists in the wild-type channel. Activation of this pathway may thus have physiological consequences in TRPM3-expressing cells such as sensory neurons. It has been reported that opening of the alternative pathway in sensory neurons increased the frequency of PS-induced spike activity in cell-attached recordings and exacerbates TRPM3-mediated pain.[44] Remarkably, heat application did not induce opening of the alternative pathway, which suggests that this pathway does not contribute to the regular process of thermosensing. However, it is possible that under specific (patho)physiological conditions an endogenous activator of both ion permeation pathways is secreted. The activating effect of this endogenous activator on TRPM3 could be temperature- dependent, which could induce a TRPM3-induced hypersensitivity toward higher temperatures.

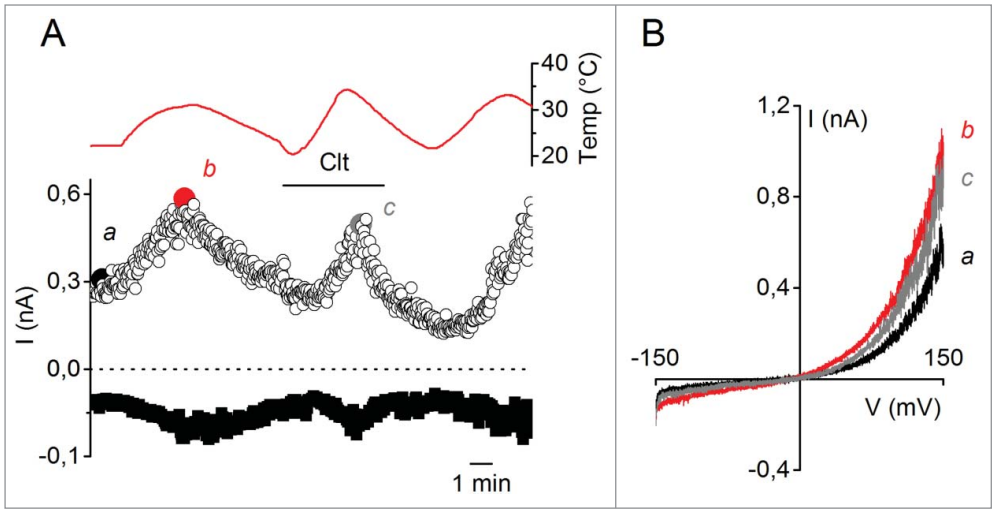

Figure 2. The modulator clotrimazole is without effect on heat-activated TRPM3 currents. (**A**) Time course of whole-cell TRPM3 expressing HEK293 cell currents at ±80 mV during heating. At the indicated time point 10 μM clotrimazole (Clt) is added. (**B**) Current (I) -Voltage (V) relations were obtained at the indicated time points in panel (**A**).

Pharmacological Properties and Modulation

Activators

Physical stimuli - TRPM3 was originally identified as a volume-regulated channel, as the channel is activated by hypotonic cell swelling.[19,22] Application of hypotonic extracellular solution produced a rise of $[Ca^{2+}]_i$ in TRPM3-transfected HEK cells, which was reversible upon reapplication of an isotonic solution. So far, the biophysical mechanism of TRPM3 activation by hypotonicity has not been investigated further, leaving the potential physiological role of TRPM3 as a volume-regulated ion channel open.

Recently, TRPM3 has been shown to be temperature-sensitive, with robust responses to heat (40°C) stimulation in TRPM3 expressing HEK293 cells (**Fig. 1**).[18] Thermal sensitivity was confirmed in whole-cell patch clamp recordings of TRPM3-expressing HEK cells. Repetitive applications of an identical heat stimulus resulted in partly desensitizing responses.[18] The endogenously expressed TRPM3 channel in nociceptive DRG and TG was also steeply activated by heating and underlies heat sensitivity in a subset of sensory neurons.[18] A further analysis of the temperature sensitive properties of TRPM3 is discussed below.

Endogenous agonists - Sphingolipids, a class of cell membrane lipids produced by the human body, were described as the first ligands to activate the human TRPM3.[54] In Fura2-based Ca^{2+}-microfluorimetry measurements, stimulation with D-erythro-sphingosine (DeSPH) specifically induced an increase in $[Ca^{2+}]_i$ in TRPM3-transfected HEK cells. The structurally related N,N-dimethyl-DeSPH and dihydro-DeSPH, also induced increases in $[Ca^{2+}]_i$.[54] A later study noticed an only minimal early effect of DeSPH on mouse TRPM3, followed by activation of a larger, linear current that was also observed in untransfected HEK293 cells.[47] The reason of this discrepancy is not clear, but can possibly be explained by the use of different TRPM3 isoforms with different biophysical properties. An overview of TRPM3 agonists is presented in **Table 1**.

The neurosteroid pregnenolone sulfate (PS) induces rapid and reversible activation of TRPM3, both in overexpression systems and in cells endogenously expressing TRPM3,[18,32,47,55,56] and is currently the most potent TRPM3 agonist described in the literature. In patch clamp experiments, stimulation of HEK293 cells stably expressing TRPM3 induced an outwardly rectifying current (EC50 ~23 μM at room temperature (RT)).[47] PS is a substance produced in considerable amounts by the human body

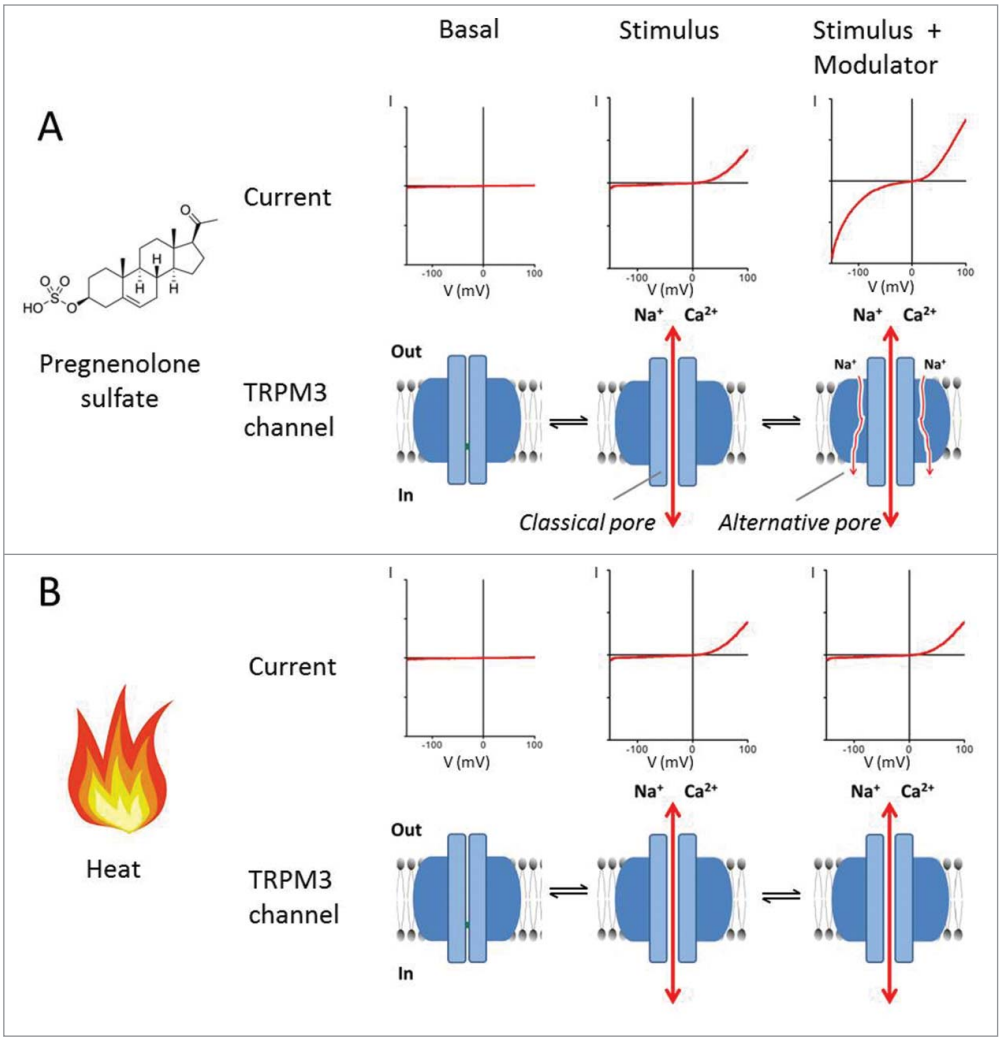

Figure 3. Biophysical gating properties of TRPM3. (**A**) Pregnenolone sulfate-activated TRPM3 currents are outwardly rectifying but develop an inwardly rectifying component in response to a combination of the agonist (PS) and the channel modulator clotrimazole (Clt). The inwardly rectifying component is not carried by the central pore and is likely to be carried by an alternative ion permeation pathway. (**B**) Heat-activated TRPM3 currents are outwardly rectifying and incubation with Clt was without effect. Heat stimulation induces the opening of the central pore.

([plasma] ~100 – 800 nM), although the conditions under which elevated PS levels may gate TRPM3 are not known.[57] Interestingly, increasing the temperature from RT to 37°C strongly sensitizes TRPM3 for PS, and it is has been proposed that the PS concentrations encountered physiologically in the human body may be sufficient to activate TRPM3 channels, especially at the body core temperature.[18,47,58] The closely related substances pregnenolone, dehydroepiandrosterone (DHEA), and DHEA sulfate also activate TRPM3, but with a reduction in potency or efficacy.[47] Several other, closely related steroidal analogs of PS have been identified to activate TRPM3.[45,59]

Small-molecule exogenous agonists - The 1,4-dihydropyridine nifedipine quickly and reversibly activates TRPM3.[47] It has been argued that PS and nifedipine act on TRPM3 via separate binding sites, since co-application of PS and nifedipine caused a larger activation of TRPM3 than applying these compounds alone, even at the highest concentration of PS.[45]

Table 1. Agonists of TRPM3 channels

Class of substance	Substance	EC_{50} [M]	Ref
Sphingolipid	D-erythro-sphingosine	12×10^{-6}	51
	N, N-dimethyl-D-erythro-sphingosine	n.d.	51
	dihydro-D-erythro-sphingosine	n.d.	51
Steroid	pregnenolone sulfate	23×10^{-6}	44
	pregnenolone	15×10^{-6}	44
	Dehydroepiandrosterone (DHEA)	62×10^{-6}	44
	DHEA sulfate	299×10^{-6}	44
	epiallopregnanolone sulfate	14×10^{-6}	42
1, 4-dihydropyridine	Nifedipine	30×10^{-6}	44

Blockers

Endogenous blockers – TRPM3 channels are partially inhibited by cholesterol, and by steroids such as progesterone, dihydrotestosterone, pregnanolone, 17 OH-progesterone, 21 OH-progesterone and estradiol.[32,46] Further investigation is required to examine the physiological impact of these partial blockers on endogenously expressed TRPM3.

Exogenous blockers - The peroxisome proliferator-activator receptor - γ (PPAR-γ) agonists rosiglitazone, troglitazone and pioglitazone were identified as the first exogenous inhibitors of PS-induced Ca^{2+} influxes in TRPM3 expressing HEK cells.[60] TRPM3 channels can also be blocked by fenamates, such as mefenamic acid.[55] Other fenamate structures like DCDPC, flufenamic acid, meclofenamic acid, and tolfenamic acid are all non-selective blockers of TRPM3.[55] In addition, 5 natural compounds of the citrus fruit flavanones were identified as more potent and selective blockers, of which isosakuranetin and liquiritigenin are currently the most potent inhibitors of TRPM3.[56] Other flavanones that are able to inhibit TRPM3 currents include naringenin, eriodictyol and hesperetin.[61] These compounds exhibited a marked specificity for recombinant TRPM3 compared to other TRP channels, and blocked PS-induced $[Ca^{2+}]_i$ signals in freshly isolated DRG neurons.[61] In addition,

the deoxybezoin ononetin was also identified as a selective and potent TRPM3 blocker.[56]

In addition, currents mediated by TRPM3 can be inhibited by the TRPM3-specific polyclonal antibody (TM3E3), and by several synthetic compounds including the calmodulin antagonist W-7 and econazole.[32,44,62] Additionally, TRPM3 can be blocked by highly nonspecific blockers, such as 2-APB[63] and the trivalent lanthanides Gd^{3+} and La^{3+}.[19,22,54] An overview of TRPM3 antagonists is presented in **Table 2**.

Modulators

At the moment, very little is known about intracellular modulators of TRPM3. A Ca^{2+}- dependent binding of calmodulin to the putative calmodulin binding site at the N-terminus of TRPM3 has been shown biochemically.[64] Interestingly, S100A1 binds to the same regions, which may indicate a dual regulation of TRPM3 channels by calmodulin/S100A1. Finally, PtdIns (4,5)P2 also interacts with a CaM/S100A1 binding site implying a central role of these regions for the regulation of TRPM3.[64] However, this interaction was only observed with purified channel fragments, and it is unclear whether these interactions also occur in intact, full-length channels. To better understand the (patho)physiological role of TRPM3 in sensory neurons further investigation is required to indicate the biophysical impact of these and other modulators on the TRPM3 activity.

Mechanism of Thermosensation in TRPM3

In the literature, temperature dependence of TRP channels is often described by thermal threshold ($T_{threshold}$) values and Q_{10} values. The term thermal threshold is vaguely defined as the temperature at which a thermosensitive channel shows first activation. However, since thermosensitive gating of a channel is not an all-or-nothing event as in the case of action potentials but rather a gradual process, $T_{threshold}$ mainly reflects the temperature at which one can distinguish between current and background noise, which is strongly dependent on experimental settings and quality.[65] Therefore, the use of $T_{threshold}$ may lead to confusion and should be abandoned, and comparing thermal thresholds of TRPM3 with those of other thermoTRPs is meaningless. A Q_{10} value is classically defined as the ratio of a reaction rate (α) measured at 2 temperatures 10 degrees apart:

$$Q_{10} = \frac{\alpha_{T+10}}{\alpha_T}, \quad (1)$$

where T stands for the temperature. This expression can be generalized to obtain the Q_{10} value from measurements at any 2 temperatures:

$$Q_{10} = \left(\frac{\alpha_{T2}}{\alpha_{T1}}\right)^{\frac{10}{T_2 - T_1}} \quad (2)$$

In the field of ion channel research, current amplitudes (I) are often used instead of reaction rates, which leads us to the equation (3).

$$Q_{10} = \left(\frac{I_{T2}}{I_{T1}}\right)^{\frac{10}{T_2 - T_1}} \quad (3)$$

In the case of TRPM3, a Q_{10} value of 7.2 has been determined,[18] which is high in comparison with typical ion channels (Q_{10} ~3),[66] but relatively low compared to reported values for some other thermoTRPs (e.g. TRPV1 with $Q_{10} > 15$).[18,67-69] However, also Q_{10} values should be interpreted carefully. First, current amplitudes do not show a linear behavior if plotted in an Arrhenius plot. Therefore, the definition of a single Q_{10} value is not possible. Often researchers give 2 Q_{10} values, reflecting tangent fits for the low and the high temperature parts of the plot. However, like $T_{threshold}$, these values are highly depending on the ratio between currents carried by the channel of interest and background currents.[65] Moreover, the Q_{10} value by itself is not a single thermodynamic value, but reflects the influence of temperature (1) on the number of ion channels expressed at the cell membrane, (2) on the ionic flux through an open channel pore, and (3) on P_{open}, the mean open probability of the channel. As such, Q_{10} values determined from current amplitudes represent the influence of temperature on all 3 parameters:

$$Q_{10} = Q_{10,expression} \times Q_{10,flux} \times Q_{10,gating} \quad (4)$$

Since the number of channels in the plasma membrane or the ion flux through a single open channel pore are mostly only mildly influenced by temperature, steep temperature dependence of thermosensitive channels is mainly determined by $Q_{10,gating}$. As outlined below, $Q_{10,gating}$ can be related to relevant

Table 2. Inhibitors of TRPM3 channels

Class of substance	Substance	IC_{50} [M]	Ref
Steroid	Cholesterol	1×10^{-3}	29
	Progesterone	10×10^{-6}	43
	Dihydrotestosterone	50×10^{-6}	43
	Pregnanolone	n.d.	43
	17 OH-progesterone	n.d.	43
	21 OH-progesterone	n.d.	43
	Estradiol	n.d.	43
PPAR-γ	Rosiglitazone	$5–10 \times 10^{-6}$	57
	Troglitazone	12×10^{-6}	57
	Pioglitazone	12×10^{-6}	57
Fenamates	Mefenamic acid	6.6×10^{-6}	52
	DCDPC	7.5×10^{-6}	52
	Flufenamic acid	33.1×10^{-6}	52
	Meclofenamic acid	13.3×10^{-6}	52
	Tolfenamic acid	11.1×10^{-6}	52
Flavanone	Isosakuranetin	50×10^{-9}	53
	Liquiritigenin	0.5×10^{-6}	53
	Naringenin	0.5×10^{-6}	58
	Eriodictyol	1×10^{-6}	58
	Hesperetin	2×10^{-6}	58
DeoybezoinOthers	Ononetin	0.3×10^{-6}	58
	W-7	15×10^{-6}	59
	Econazole	6×10^{-6}	41
	La^{3+}, Gd^{3+}	n.d.	16, 19

thermodynamic parameters when gating models are used to describe the temperature dependence of thermoTRPs.

Models of thermosensitive gating in TRP's

Several studies in the past aimed at understanding the molecular and biophysical basis of thermosensitivity in TRP channels, and 2 major competitive views of how temperature influences gating of thermoTRPs have emerged. One view explains the thermosensitivity of TRP channels based on simple thermodynamic rules,[70,71] where temperature directly affects the equilibrium between the open and closed state(s) of the channel, based on global changes in enthalpy and entropy during channel gating. In its simplest form, this can be expressed as a 2-state model, which has been used to describe gating of several thermoTRPs.[18,71–73] Clearly, a simple 2-state model is a simplification of the actual physiological processes as for instance single channel recordings show that thermoTRPs exhibit multiple open and closed states.[67,74]

A second view is the allosteric model,[75–78] where thermosensitivity is determined by a limited number of thermosensing modules, comparable to the voltage-sensing modules in voltage-gated ion channels, which transit between an active and inactive state depending on temperature. The activation state of the thermosensor modules influences the equilibrium between the close and open pore, and the strength of this influence is determined by an allosteric coupling constant. Although appealing, there is currently little evidence for the existence of a limited number of delineated thermosensor modules. In contrast, several studies show that mutagenesis in multiple distal regions can influence temperature sensitivity of TRP channels,[68,79–81] and, obviously, temperature will affect every single atom in the channel complex.

Since the temperature dependence of TRPM3 gating can be accurately described using a simple 2-state model, we briefly describe the properties of this model below. It should be noted, as demonstrated elsewhere,[65] that the 2-state model actually presents a specific situation of the allosteric model, namely where coupling between thermosensing module(s) and pore opening is very strong, which implies that a channel fully closes and maximally opens at extreme temperatures.

The Two-State Model

Consider a channel with one open and one closed state, with gating transitions described by the opening rate α and the closing rate β:

$$C \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} O.$$

In thermodynamics, the equilibrium between such 2 states can be described as

$$K_{eq} = \frac{O}{C} = \frac{\alpha}{\beta} = e^{-\frac{\Delta G}{RT}}, \quad (5)$$

where ΔG is the difference in Gibbs free energy between the 2 states. ΔG is related to the differences in enthalpy (ΔH) and entropy (ΔS)

$$\Delta G = \Delta H - T\Delta S - E, \quad (6)$$

where E describes any other form of energy that has an effect on the equilibrium between O and C. Several thermoTRPs are voltage-gated, in which case E equals zFV, where z is the gating charge, F the Faraday constant and V the transmembrane voltage. With this information P_{open} can be calculated as follows.

$$P_{open} = \frac{O}{O+C} = \frac{1}{1+\frac{1}{K}} = \frac{1}{1+e^{\left(\frac{\Delta H - T\Delta S - zFV}{RT}\right)}} \quad (7)$$

At low open probabilities, $Q_{10,gating}$ can be related to ΔH

$$Q_{10,gating} = 10^{\frac{10}{\ln 10}\times\frac{(\Delta H - zFV)}{RT^2}} = e^{10\times\frac{(\Delta H - zFV)}{RT^2}}. \quad (8)$$

More detailed kinetic information on the gating behavior can be obtained by actually determining the temperature dependence of the opening and closing rates α and β, which can be obtained experimentally from current relaxation time constants when abruptly changing the equilibrium (e.g., by a change in voltage or temperature). These rates are related to the enthalpies and entropies associated with channel opening and closing, according to:

$$\alpha = \frac{k_b T}{h} \times e^{\frac{-\Delta H_{open} + T\Delta S_{open}}{RT}} \times e^{\frac{\delta zFV}{RT}} \quad (9)$$

$$\beta = \frac{k_b T}{h} \times e^{\frac{-\Delta H_{close} + T\Delta S_{close}}{RT}} \times e^{-\frac{(1-\delta)zFV}{RT}} \quad (10)$$

where k_b is the Boltzmann constant ($1.38\dot{c}10^{-23}$ J/K), h the Planck constant ($6.63\dot{c}10^{-34}$ J/s) and δ the fraction of gating charge moved in the outward direction between the closed state and the transition state. Note that $\Delta H = \Delta H_{open} - \Delta H_{close}$ and $\Delta S = \Delta S_{open} - \Delta S_{close}$.

Application of the 2-state model to TRPM3

Using the experimentally determined values for TRPM3 $\Delta H_{open} = 138$ kJ mol^{-1} $\Delta H_{close} = 12$ kJ mol^{-1} $\Delta S_{open} = 258$ J mol^{-1} K^{-1} $\Delta S_{close} = -120$ J mol^{-1} K^{-1} and z= 0.55 [18] the thermal behavior of the channel can be modeled. Comparison with actual current measurements indicates that the 2-state model can accurately predict the temperature dependence of TRPM3 gating (**Fig. 4A and B**).

Moreover, this analysis allows a more robust comparison of the temperature sensitivity of TRP channels than for instance Q_{10} or thermal thresholds. For instance, based on these

parameters, a temperature of half activation (T_{50}) can be determined as

$$T_{50} = \frac{\Delta H - zFV}{\Delta S}. \qquad (11)$$

At a membrane potential of −60 mV, this analysis yields T_{50} = 60.0°C for TRPM3 compared to T_{50} = 47.5°C for TRPV1. This suggests that TRPM3 is acting in a higher temperature range than TRPV1, which can also be visualized by plotting the modeled mean open probability of both channels at −60 mV in function of temperature (**Fig. 4C**). The T_{50} value for TRPV1 corresponds remarkably well with the published value of 46.1°C, which was obtained by microfluorimetric Ca^{2+} imaging.[82] Although T_{50} for TRPM3 has not been experimentally determined, available experimental data indeed indicates that the current-temperature relation of the inward TRPM3 currents is shifted to higher temperatures compared to TRPV1.[18] Since TRPM3 and TRPV1 share several features, including the expression pattern in sensory neurons, ion permeability and activation by heat, it can be speculated that they partly have overlapping physiological functions as temperature sensor for noxious heat signals in the somatosensory system, where TRPM3 might become more prominent at higher temperatures.

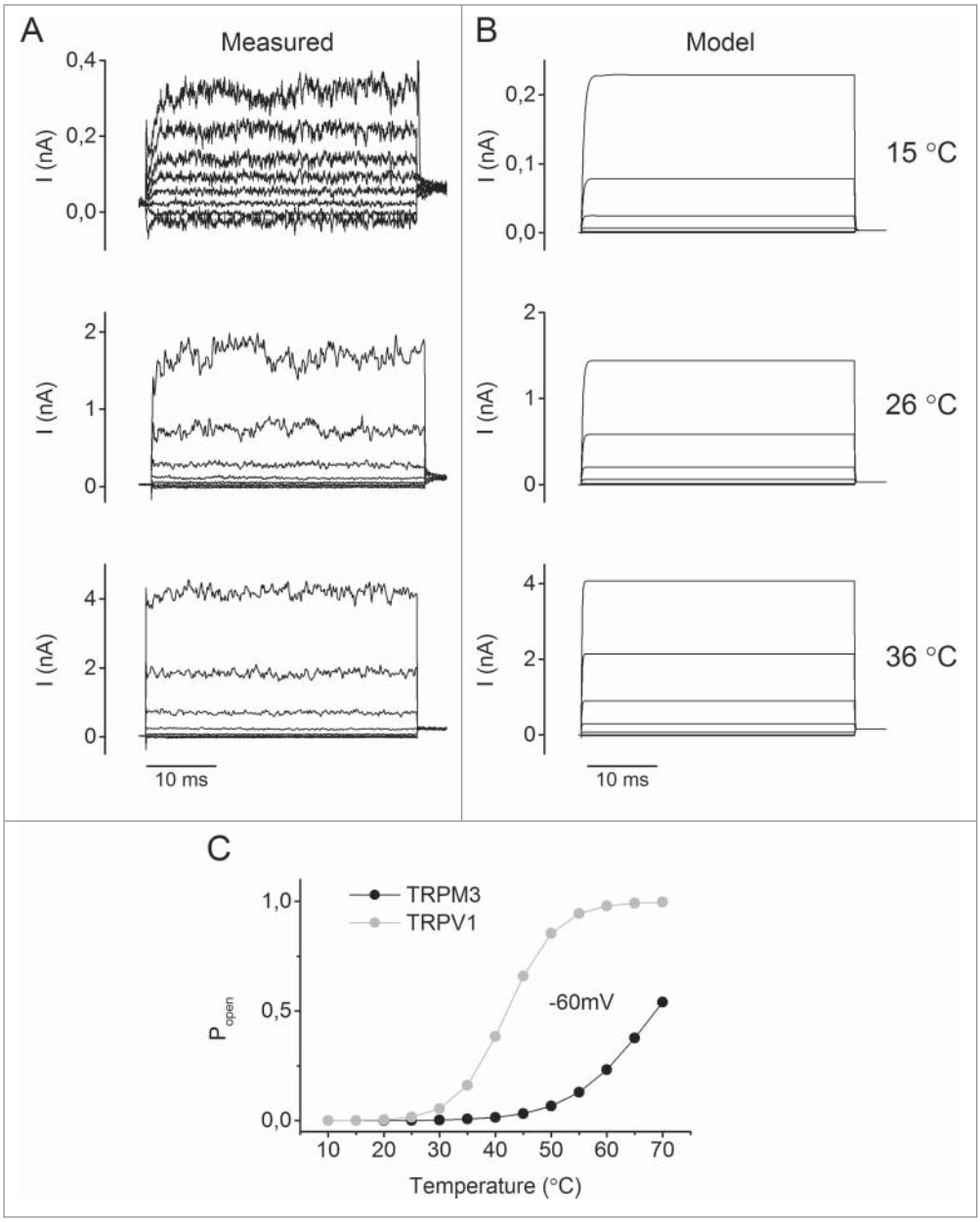

Figure 4. Modeling of TRPM3 heat sensitivity. (**A**) Experimentally obtained current traces of TRPM3 measured with whole-cell patch clamp recordings in HEK293T cells. A step protocol was applied ranging from –80 mV to +200 mV in steps of 40 mV. The measurements presented were recorded at 3 different temperatures (15°C, 25°C and 26°C). (**B**) Modeled current traces obtained by applying a 2-state model together with the same step protocol and the same temperatures as in (**A**). (**C**) Open probabilities of TRPM3 and TRPV1 obtained by applying the 2-state model at different temperatures.

It is known from other thermosensitive TRP channels like TRPM8 or TRPV1 that, besides temperature, their gating can also be influenced by chemical ligands. In the case of TRPM8, this has been modeled using a Monod-Wyman-Changeux (MWC) model, with 4 binding sites of the ligand (i.e. one per subunit). In this model, each bound ligand results in an equivalent reduction of the enthalpy difference between the open and closed channel (ΔH). As a result, the more ligands are bound to the channel, the more the open state becomes stabilized relative to the closed state.[41,83] By applying similar considerations to the TRPM3 channel, current traces at different temperatures under influence of the agonist PS (5 μM) were modeled. Again the modeled traces showed high resemblance with the experimental data (**Fig. 5A-B**), and reproduced experimental findings that both PS and heat induce a leftward shift of the voltage-dependence of activation (**Fig. 5C**).

As illustrated above, the 2-state model can accurately predict the thermosensitivity of TRPM3, and the MWC-model further describes the synergism between heat and PS, which may be

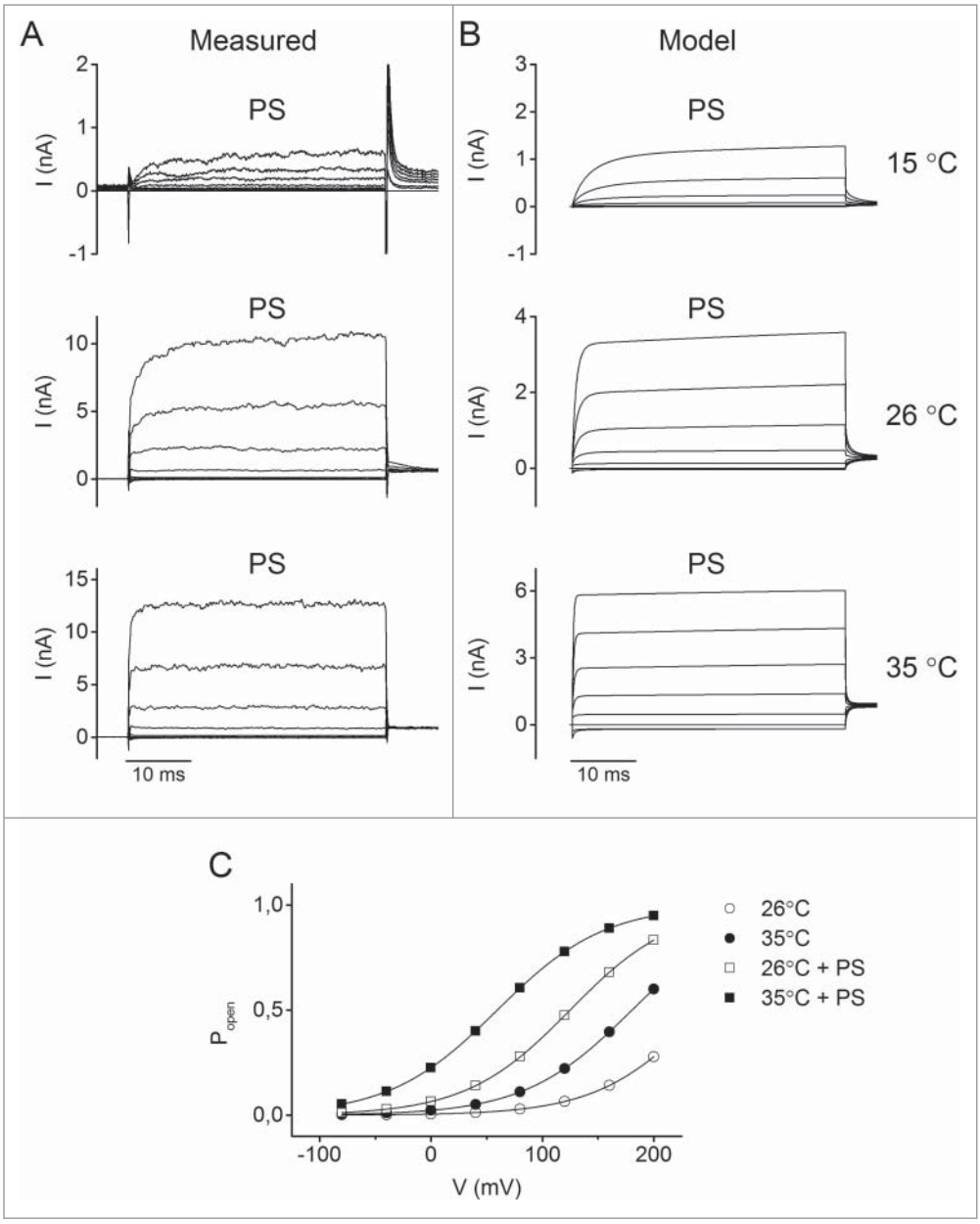

Figure 5. Synergism of heat and the TRPM3 agonist pregnenolone sulfate (PS). (**A**) Experimentally obtained current traces of TRPM3 measured with whole-cell patch clamp recordings in HEK293T cells. A step protocol was applied ranging from –80mV to +200mV in steps of 40 mV. The measurements presented were recorded at 3 different temperatures (15°C, 25°C and 26°C) in presence of 5μM pregnenolone sulfate (PS). (**B**) Modeled current traces obtained by applying a Monod-Wyman-Changeux (MWC) model together with the same step protocol, temperatures and PS concentration as in (**A**). (**C**) Modeled open propabilities of TRPM3 at different voltages for 2 different temperatures in presence and absence of 5 μM PS.

relevant in pain conditions involving increased levels of TRPM3 agonists.[44] Furthermore, the model-based comparison with TRPV1 describes in a quantitative manner that TRPM3 operates in a higher temperature range than TRPV1. Nevertheless, the fact that available experimental results are readily described by the 2-state model does not exclude an allosteric mechanism, as the 2-state model actually presents a specific situation of the allosteric model.[65] Future experiments, preferentially involving detailed kinetic and/or single-channel analysis at extreme temperatures and voltages, may provide more details on the degree of coupling between the thermal/voltage stimuli and channel opening in TRPM3, and thus on the validity of these or other models.

Physiology and Potential as a Therapeutic Target

Physiological function of TRPM3

A first physiological function of TRPM3 was described in the pancreas, where the channel is functionally expressed in insulin-secreting β-cells.[47] Stimulating pancreatic β-cells with high doses of PS (50 μM) increased glucose-induced insulin release.[47] It was suggested that TRPM3 is an essential component of an ionotropic steroid receptor enabling unanticipated crosstalk between steroidal and insulin-signaling endocrine systems.[47] However, no obvious effects are observed in the resting blood glucose level in TRPM3-deficient mice, which show no signs of developmental or metabolic deficits.[18] Altogether, only a minor role for TRPM3 in normal glucose-induced insulin release is proposed, although it cannot be excluded that TRPM3 modulates insulin release under specific (patho)physiological conditions.[37]

Later reports indicate a role of TRPM3 as a chemo- and heat sensor in the somatosensory system. TRPM3 proteins are expressed in a small subset of nociceptor neurons of the somatosensory system in mice and form PS-activated channels with a similar pharmacology and biophysical properties as heterologously expressed channels.[18] Importantly, the number of PS- sensitive neurons is strongly reduced in TRPM3-deficient mice. Intraplantar injection of PS induces nocifensive behavioral responses like licking or lifting of

the injected paw, which are lacking in TRPM3 deficient mice.[18] Moreover, in vivo application of the TRPM3 specific inhibitors, isosakuranetin and hesperetin, significantly reduce the sensitivity of mice to PS-induced chemical pain.[56] All together these arguments suggest for a possible role of TRPM3 as a chemo-sensitive nociceptor.

Additionally, TRPM3 is steeply activated by heating and underlies heat sensitivity in a subset of sensory neurons. The subgroup of heat-sensitive neurons responding to PS but not to capsaicin is strongly reduced in TRPM3 deficient mice.[18] TRPM3-lacking mice exhibit clear deficits in their avoidance responses to noxious heat, as evidenced by prolonged reaction latencies in the tail immersion and hot plate assays and a reduced avoidance of hot temperature zones in the thermal gradient and thermal preference tests. The difference in heat responsiveness between wild-type and TRPM3-deficient mice is even more pronounced following injection of complete Freund's adjuvant. This inflammatory challenge causes a significant reduction in the response latencies in wild-type mice but do not change the heat response latencies in TRPM3$^{-/-}$ mice. Similarly, pharmacological inhibition of TRPM3 using citrus fruit flavanones reduces the sensitivity of mice to noxious heat, notably without altering their body temperature.[56] Taken together, these findings establish TRPM3 as a thermosensor in the somatosensory system, involved in the detection of noxious stimuli in healthy and inflamed tissue.[18] However, further *in vitro*, single-fiber action potential recordings from C-fibers are required to assess the role of TRPM3 as heat sensor.

Furthermore, recent studies in the literature suggest a possible role for TRPM3 in regulating pupil constriction.[26,30,84] In addition, TRPM3 channels have been implicated in rheumatoid arthritis,[85] blood vessel contraction, proliferation of smooth muscle cells[32] and in hypo-osmolality-induced ductus arteriosus contraction.[86] However, no noticeable phenotype in this respect was observed in TRPM3-deficient mice.

Potential of TRPM3 as a therapeutic target for chronic pain

The elucidation of the essential role of temperature-sensitive TRP channels in various forms of acute and chronic pain has created the opportunity to investigate their potential as molecular targets for novel, more specific analgesic drugs.[87] TRPM3 is identified as nociceptor channel involved in acute heat sensing and inflammatory heat hyperalgesia, and thus as a potential target for analgesic treatments.[18] Indeed, *in vivo* application of the TRPM3 inhibitors isosakurentin and hesperetin significantly reduced the nociceptive effect on heat or PS-induced pain responses.[56] In rats with chronic constriction injury -induced neuropathic pain, it was shown that liquiritigenin is extreme efficacious in attenuating the thermal, mechanical and cold hyperalgesia.[88] One has to be careful with the interpretation of these results as liquiritigenin is a selective and potent estrogen receptor β (ER-β) agonist[89] and a potent inhibitor of CYP19.[90] The effects of liquiritigenin on P450 enzymes and its ER-β-agonistic properties may interfere with its TRPM3-blocking properties in vivo. The discovery and development of TRPV1 blockers and analgesics has been hampered by the severe side effect of hyperthermia related to TRPV1 blockade and the enthusiasm of targeting TRPV1 for the management of pain has been dampened. In contrast to TRPV1 blockers, systemic administration of TRPM3 blockers does not induce significant alterations in body core temperature.[56] Furthermore, injection of the TRPM3 agonist PS does not induce hypothermia,[18] which represents a striking difference between TRPM3 and TRPV1.[3] All together these results raise the possibility that TRPM3 may be a superior analgesic drug target, lacking the adverse effects on core body temperature observed with most TRPV1 blockers. One explanation for the observation that in vivo inhibition of TRPM3 using antagonists has no effect on core body temperature, as opposed to TRPV1 antagonists, may lie in biophysical properties of the channels. Indeed, as pointed out above, TRPM3 is activated at significantly higher temperatures than TRPV1, and may thus have a much smaller contribution to thermosensory/thermoregulatory processes that occur at normal body temperature.[91] In that way TRPM3 is only involved in nocifensive responses to heat and not in autonomic thermoregulation. In agreement with this, TRPA1 may be involved in nocifensive responses to cold in some species,[72,92-95] but is not a cold sensor for autonomic thermoregulation in rodents.[96] This would be in contrast to the TRPM8 channel that is a physiologically important thermosensor for the thermoregulation system. TRPM8 antagonists cause hypothermia in rats and mice,[97,98] and the magnitude of the hypothermic response increases with a decrease in the ambient and body temperatures. An alternative explanation may lie in a different expression pattern between TRPV1 and TRPM3. Key brain centers involved in controlling the body core temperature are the preoptic area and anterior hypothalamus, where expression of TRPV1 has been suggested.[99,100] At the moment the knowledge related to TRPM3 expression in the human brain is very limited.[24] Moreover, important thermosensory information originates from visceral sensory neurons, and currently no knowledge is available about the functional TRPM3 expression in visceral sensory neurons. A further investigation of the exact expression pattern of both proteins might lead to a better understanding of the possible impact on the regulation of the body core temperature of these molecules. Besides the strong candidacy of TRPM3 as therapeutic target for chronic pain, future work will clarify the possible consequences of long term TRPM3 inhibition.

Pathophysiology of TRPM3

Until now, no inherited human disease related to the chemo- and thermosensory role of TRPM3 has been described. Very recently, the first human disease linked with *Trpm3* was described in patients with hereditary eye disease. A heterozygous A-to-G transition in exon-3 of *Trpm3* gene was associated with inherited ocular disease in humans, including inherited cataract and high-tension glaucoma with variable anterior segment defects.[101] Since *Trpm3* expression is described in the ciliary body, which co-operates in the

production of aqueous humor,[102] it is possible that *Trpm3* may influence the intra-ocular pressure. Outside the ciliary body, the expression of *Trpm3* in the lens epithelium and retinal pigment epithelium may suggest that the channel could participate in the maintenance of intracellular Ca^{2+}, essential for preserving lens transparency, and in the regulation of Ca^{2+} fluxes in the sub-retinal space that accompany light/dark transitions.[103,104]

Concluding Remarks and Further Perspectives

The role of TRPM3 as a sensor of ambient warm to hot temperatures is being established. TRPM3 is involved in the detection of noxious heat, similar as was described for TRPV1. Both TRPV1 and TRPM3 are heat-sensitive ion channels expressed in DRG neurons, and contribute to thermal pain sensation. However, pharmacological inhibition of TRPV1 in the TRPM3-deficient mice do not fully abrogate avoidance responses to noxious heat,[18] implying the existence of additional mechanisms for sensing noxious heat. TRPM3 also appears to play an important part in chronic pain, causing, for instance heat hypersensitivity or hyperalgesia under conditions of nerve injury and inflammation. However, we still need more details about the nature of TRPM3s role in various pain states in order to effectively guide drug discovery and therapeutic strategies. Moreover, its role in other cell types and tissues is poorly understood, which makes it difficult to predict the consequences of long-term TRPM3 antagonism. The potential of TRPM3 as a viable disease target for therapeutic intervention rests on our further understanding of the role of this channel in both normal and diseased states, as well as on the ability of therapeutic molecules to achieve a fine balance between efficacy and toxicity.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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