Chapter 6
TRPC2: Of Mice But Not Men

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Abstract Relatively little is known in regard to the physiological significance of TRPC2 and its regulation or interaction with other calcium regulating signalling molecules. In rodents, however, the importance of TRPC2 is indisputable. In mice, transcripts for TRPC2 have been found in testis, sperm, in neurons in the vomeronasal organ, and both in the dorsal root ganglion and in the brain. In rats, TRPC2 is thought to be expressed exclusively in the vomeronasal organ. In mice, TRPC2 is of importance in regulating both sexual and social behaviour. In sperm, TRPC2 is of importance in the acrosome reaction. This review will summarize the known physiological effects of TRPC2 channels, and the regulation of the function of the channel. In addition, some new preliminary data on the role of TRPC2 in rat thyroid cells will be presented.

6.1 Introduction

Of all the different members of the TRPC – family of ion channels, the TRPC2 channel is perhaps the least investigated, and thus relatively little is known in regard to its physiological significance and regulation of interaction with other calcium regulating signalling molecules. This discrepancy stems from the fact that TRPC2 is a pseudogene in human, and thus has little importance in understanding human pathophysiology. However, in rodents the importance of TRPC2 is indisputable. In mice, transcripts for TRPC2 have been found in mice testis, sperm, in neurons in the vomeronasal organ, and both in the dorsal root ganglion and in brain. In rats, TRPC2 is thought to be expressed exclusively in the vomeronasal organ. In regard to the regulation of the acrosome reaction in sperm, TRPC2 appears to be participating in calcium signalling. Even more important, in regulating both sexual and social behaviour in mice, TRPC2 is of exceptional physiological importance. The

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knowledge regarding how the activation of TRPC2 is regulated by physiological stimuli, and information on the gating properties of the channel, is limited. Some characteristics may be deduced from comparison to other members of the TRPC family, but for understanding e.g. pheromone signalling, information regarding the regulation of TRPC2 channels is of physiological importance. This review will summarize the known physiological effects of TRPC2 channels, the known mechanisms by which TRPC2 activation and signalling is regulated, and the electrophysiological characteristics of the TRPC2 channels. For a more detailed review of the molecular biology, pharmacology and pathophysiology of TRPC2 and other members of the TRPC-family of ion channels, the reader is referred to some excellent recent reviews [1, 2].

6.2 The Regulation of TRPC2

Although TRPC2 is a pseudogene in humans, the regulation of its function converges on phospholipase C (PLC), in line with the regulation of other members of the TRPC channel family (reviewed in [3]). TRPC2 is a rather nonselective cation channel and affect cell function through its ability to mediate both Ca^{2+}-entry, to evoke calcium-mediated signalling, and Na^{+}-entry to collapse the plasma membrane potential. Furthermore, of all the seven members of the TRPC family, TRPC2 has the most restricted pattern of expression [1].

The first report of the TRPC2 gene was by Zhu et al. [4]. Vannier et al. [5] found two splice variants of mouse TRPC2 (TRPC2a and TRPC2b) that, when heterologously expressed in COS-M6 cells, enhanced both receptor-operated calcium entry (ROCE) and store-operated calcium entry (SOCE). However, another group found two other splice variants of mouse TRPC2, mTRPC2α and mTRPC2β, which did not enhance ROCE or SOCE when expressed in HEK-293 cells, as they failed to reach the plasma membrane and were retained in intracellular membranes [6]. A later study showed that the TRPC2α splice variant was an incompletely processed transcript of TRPC2a [7], which could explain at least in part the differences in the results. Evidence supporting the results that TRPC2 is involved in ROCE and SOCE was obtained in a study in CHO cells, where Gailly et al. [8] used a TRPC2 antisense construct and reported reductions in both ROCE and SOCE. Furthermore, inward currents were increased in HEK-293 cells transiently expressing TRPC2 after stimulation of purinergic receptors with ATP [9]. We have results that support this finding. By using shRNA for TRPC2 in rat thyroid FRTL-5 cells, we saw a reduced calcium entry when stimulating purinergic receptors [10]. In sperm, it seems like TRPC2 functions as a SOC channel, since an antibody against TRPC2 decreased both thapsigargin-induced and zona pellucida (ZP3) induced calcium entry [9].

The mechanism by which TRPC2 is activated is not yet completely understood. However, Lucas et al. [11] compared diacylglycerol (DAG) induced inward currents in wild-type and TRPC2 knockout vomeronasal sensory neurons, and found that TRPC2 knockout severely reduced the inward current. Interestingly, they reported no activation of TRPC2 in response to store depletion or increased levels of IP_3.

The involvement of TRPCs in SOCE is debatable, but it is intriguing that the master regulator of SOCE, Stim1, binds to TRPC2 and several of the TRPCs via its ezrin/radixin/moesin (ERM) domain [12].

TRPC2, like all other TRPCs, have a binding site for calmodulin (CaM) in the C-terminus [13]. In addition, the long N-terminus of TRPC2 has binding sites for CaM [7], enkurin [14] and ankyrin (see [15]). The CaM/IP_3 receptor-binding (CIRB) domain that is conserved in all TRPCs is also present in mouse TRPC2 [13]. CaM competes with the IP_3 receptor (IP_3R) in a calcium-dependant manner for binding to the CIRB domain [13], indicating that calcium through binding to CaM could mediate a negative feedback loop. Accordingly, currents through TRPC2 were inhibited by calcium-CaM in vomeronasal sensory neurons [16].

Different adaptor proteins have been shown to interact with TRPCs and IP_3Rs. One such adaptor is Homer 1, which co-immunoprecipitates with TRPC2 [17]. Homer 1 does not only function as a scaffold for TRPCs and IP_3Rs, it inhibits spontaneous activity of TRPC1 [17]. Furthermore, Homer 1 controls the translocation of TRPC3 to the plasma membrane in response to store depletion or stimulation of the IP_3R [18]. In agreement with these findings, Homer 1 knockout mice show perturbations in skeletal muscle function. In myotubes from these mice the basal current density and spontaneous cation influx was increased [19]. Another system where TRPC2 has reported functions is in erythroblasts. Stimulation of the erythropoietin (EPO) receptor activates, via PLCγ, calcium entry through TRPC2 [20]. A signaling complex between the EPO receptor, TRPC2, PLCγ, and IP_3R2 was shown to form in erythroblasts and in an overexpression system [21]. A complex between TRPC2 and IP_3R3 was also previously reported to form in the rat VNO [22]. In contrast to an earlier report [23], Chu et al. [24] showed that TRPC2 could form complexes with TRPC6 in erythroblasts.

6.3 Electrophysiological Properties of TRPC2

The notion that TRPC2 protein are highly expressed in murine vomeronasal organ (VNO) neurons suggested that it might constitute a channel involved in the transduction cascade of olfaction and opened a possibility to study the protein function in an native cellular environment [25]. VNO neurons have bipolar characteristics; single apical dendrites extend towards the surface of the sensory epithelium, which is delineated by the dendritic microvilli that contain the transduction machinery, and axons that project towards the accessory olfactory bulb (or vomeronasal bulb) [26]. These cells harbour unique sets of G-protein coupled receptors, derived from two receptor families (VR1 and VR2) suggested to be sensitive to pheromones [27–29]. Neurons having soma closer to the apical border of the sensory epithelium express receptors of type VR1 and $G_{\alpha i2}$ subunits, whereas cell bodies near the basal region contain VR2 receptors and $G_{\alpha o}$ subunits, indicating an existence of topographically and functionally segregated transduction systems [30].

VNO neurons exhibit a relatively high input resistance (several GΩ), which renders their membrane voltage (V_m) very sensitive to small fluctuations in membrane

currents [31–33]. Apparently the preparation procedure may affect the resting V_m, as the isolated cells tend to be more depolarised [31–33] compared with those embedded in slices [34, 35].

When exposed to diluted urine or putative pheromones, the firing rate of VNO neurons increases robustly. The pheromone-evoked spiking depends on PLC activity and is associated with an increase in intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) [36, 37]. The Ca^{2+} influx is initiated at distal dendrites [37] containing the sensory microvilli that show abundant TRPC2 expression [25]. Multiunit microelectrode array recordings of VNO neuroepithelium [38], or on-cell patch clamp and field potential recordings done with VNO slices [39], revealed that neurons obtained from TRPC2–/– mice are unresponsive to urine derived pheromones, confirming that TRPC2 is an essential component of olfactory transduction.

The pheromone-activated channel containing the TRPC2 protein is directly activated by DAG, the level of which is increased by PLC activity and reduced by diacylglycerol kinase (DGK) [11]. Brief stimulations of inside-out membrane patches (ripped from distal dendritic tips of VNO neurons) with the DAG analogue 1-stearoyl-2-arachidonoyl-*sn*-glycerol (SAG) evoked a marked increase in single-channel activity. The observed −3.3 pA single-channel current recorded at −80 mV in a symmetrical 150 mM Na^+ solution gave a slope conductance of 42 pS. The relative permeability of ions (P_{ion}) under bi-ionic conditions (as described [40]), gave P_{Ca}/P_{Na} and P_{Cs}/P_{Na} ratios of 2.7 ± 0.7 and 1.5 ± 0.3, respectively, when Na^+ was the only cation on the extracellular side. Inward whole-cell currents of VNO neurons were reduced upon replacement of Na^+ by $NMDG^+$, indicating that sodium acts as the main charge carrier. The amplitude of the TRPC2-dependent whole-cell currents was potentiated by more than twofold upon reduction of extracellular Ca^{2+} from 1 to 0.1 mM, suggesting a partial block of channels by permeating Ca^{2+} ions. Under whole-cell conditions, the TRPC2-dependent current emerged in response to DGK inhibitors, arguing for a reasonable DAG production by endogenous PLC activity. In line with this, the current evoked by DGK inhibitors was sensitive to PLC inhibitors, unlike the currents induced by application of exogenous SAG [11]. The SAG-activated currents are rapidly suppressed by a $[Ca^{2+}]_i$-and CaM-mediated feedback loop, suggesting an adaptation of the chemosensory transduction process [16], but see [36]).

A subset of VNO neurons seems to contain urine- and intracellular Ca^{2+}-activated Ca^{2+} influx routes independent of TRPC2 [16, 41–43]. The application of arachidonic acid (AA) or linolenic acid induced a slow increase in an inward current recorded at –90 mV and an increase in Ca^{2+} influx [41]. The molecular identity of this AA- and Ca^{2+}-activated channel is currently unknown. Due to the relatively high intracellular Ca^{2+} required to fully activate the channel, and due to its equal selectivity for K^+ and Na^+ and low preference for Ca^{2+}, the current is often referred to as a Ca^{2+}-activated, non-selective cation current (I_{CaNS}) [16, 42, 43]. VNO neurons exhibiting I_{CaNS} may form a TRPC2-independent transduction route serving a function of its own, or then the activation of I_{CaNS} may amplify the response initiated by TRPC2 [42, 43].

The activation of a DAG-gated and TRPC2-dependent current during the chemosensory transduction of VNO is well described [11, 38, 39]. However, the

roles of other Ca^{2+} influx routes and $[Ca^{2+}]_i$-activated currents in the adaptation or amplification of pheromone-evoked signalling are less well understood. It seems that the first level of integration appears at the level of $[Ca^{2+}]_i$, the alterations of which may trigger either amplifying (I_{CaNS}) or suppressive (Ca-CaM inhibition of TRPC2) mechanisms. The common signal for both amplification and adaptation suggests that the modulation of information processing may be conducted by close interactions between TRPC2 and Ca^{2+}-sensitive ion channels and/or regulatory proteins, or by cell-specific and reciprocally activated signalling cascades. However, the options mentioned above are not mutually exclusive.

6.4 Pheromone Signal Transduction in Olfaction

In many vertebrates, olfactory cues evoked by pheromones are important in regulating both social and sexual behaviour. In terrestrial vertebrates, two classes of olfactory neurons in distinct anatomical localizations have evolved, the main olfactory epithelium (MOE) and the vomeronasal organ (VNO) (for extensive reviews, see [44] and [45]). For the detection of pheromones, the VNO seems to be of significant importance, as VNO ablation markedly changed the behaviour of the mice compared with sham-operated littermates. A breakthrough in understanding how pheromones evoke signalling in the VNO was obtained when Liman and Dulac cloned the rat TRPC2 channel, and showed that it was exclusively expressed at both mRNA and protein levels in neurons in the VNO [25]. Furthermore, these neurons project to the accessory olfactory bulb, distinctly different from the projections from the MOE [46]. When TRPC2 knockout mice were generated independently by two groups [38, 39], the importance of TRPC2 in pheromone signalling was unveiled unequivocally. The behaviour of TRPC2–/– mice differed profoundly from their wild-type and TRPC2–/+ heterozygote littermates.

The mating behaviour in TRPC2–/– males against female mice did not differ from that seen in wild-type or TRPC2–/+ males. However, when male TRPC2–/– mice encountered intruder male mice, they did not show any aggressive behaviour, instead they repeatedly tried to mount them. In addition, TRPC2–/– mice showed defects in territory marking [38, 39]. Wild type lactating female mice usually show pronounced aggressivity against intruders, but this type of behaviour was absent from female TRPC2–/– mice. Furthermore, female TRPC2–/– mice showed a deficiency in maternal behaviour, i.e. they easily abandoned their pups. In addition, female TRPC2–/– mice showed sexual behaviour similar to male mice, i.e. they tried to mount wild-type female mice in a manner similar to that seen in male mice [47].

6.5 TRPC2 as Regulator of Calcium Entry in Sperm

Observations that mRNA for TRPC2 was expressed in bovine testis, and specifically in spermatocytes, suggested that TRPC2 may have a role in sperm function

[48]. In an elegant study, Jungnickel et al. [9] showed that TRPC2 indeed was necessary for calcium entry in the sperm. In their study they showed that when sperm went through the acrosome reaction upon contact with the egg's extracelluar matrix, in particular the ZP3 glycoprotein, calcium entry dependent on TRPC2 was initiated. Furthermore, these studies clearly showed that TRPC2 was necessary for the sustained increase in calcium entry in response to ZP3. In addition, inhibiting TRPC2 also abolished the acrosome reaction. The picture is, however, complicated, as the fertility of TRPC2–/– mice is not compromised, as sperm also express both the TRPC1 and the TRPC5 channels [9]. The mechanism by which ZP3 activates TRPC2 is presently not clearly elucidated. A direct interaction between the channel and the IP_3 receptor has been suggested. In addition, TRPC2 can be activated by depleting intracellular calcium stores using thapsigargin in sperm, suggesting that TRPC2 may participate in SOCE. In HEK-cells transfected with TRPC2, the channel was activated through a receptor-mediated mechanism, possibly due to PLC-evoked breakdown of phosphatidylinositol 4,5-bisphosphate and the production of DAG [5]. However, stimulating sperm with DAG (which activates other TRPC channels) was unable to evoke the acrosome reaction [49]. Furthermore, two adaptor proteins, enkurin and junctate, may be involved in the activation and regulation of TRPC2 [14]. Thus, TRPC seems to be activated both through a receptor- and store-dependent mechanism. It is also possible that TRPC2 may be activated by slightly different mechanisms in different cell types.

6.6 TRPC2 as Mediator of Erythropoietin-Evoked Signalling

Of the few reported physiological events where TRPC2 have been reported to participate is the erythropoietin-evoked calcium signalling in murine haematopoietic cells [20]. The effect seems to be mediated through a mechanism dependent on PLCγ and IP_3-receptors [21], in a manner similar to that reported for other TRPC channels (see e.g [50]). Interestingly, TRPC2 splice variants may block calcium entry [51]. It is worth mentioning that the erythropoietin-evoked entry of calcium in human erythroid cells seems to be mediated by TRPC3 [52].

6.7 TRPC2 in Rat Thyroid Cells

We have for several years investigated calcium signalling in rat thyroid FRTL-5 cells, a well-characterized model for studying thyroid cell function. In these investigations we have recently detected a novel calcium entry mechanism dependent on a phosphatase and protein kinase A [53]. This entry mechanism was also regulated by a receptor–mediated mechanism, and was enhanced by DAG [54]. The nature of the mechanism was, however, not known. We thus performed a RT-PCR screening for

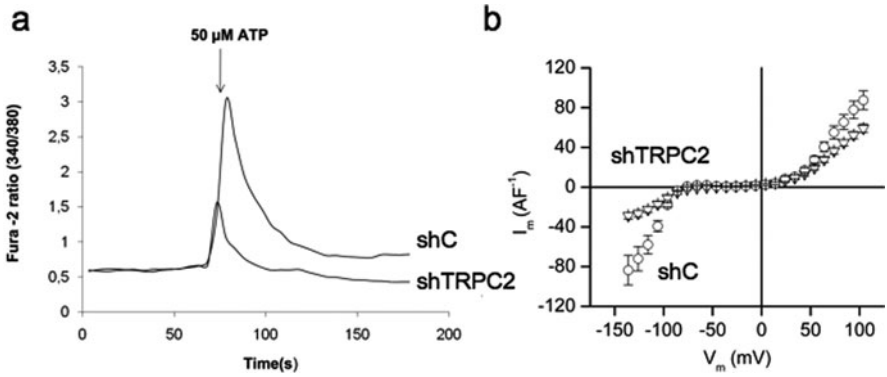

Fig. 6.1 Importance of TRPC2 for calcium signalling in rat thyroid FRTL-5 cells. (**a**). Calcium imaging studies of control cells and TRPC2 knockdown cells stimulated with ATP in calcium-containing buffer at +37°C. Each trace is representative of at least 30 separate cells. (**b**). I–V characteristics of control cells (*circle*), or TRPC2 knockdown cells (*triangle*). Whole-cell recordings were performed at +32°C in an extracellular solution containing 1.0 mM $CaCl_2$ and an intracellular solution containing 5 mM BAPTA. The voltage step protocol was initiated with a pulse to −80 mV (duration 100 ms) that was followed by a series of rectangular pulses. The series of 25 steps ascended from −120 mV to +120 mV at 20 mV increments, each pulse of 100 ms duration. Steps were applied every 2 s from a holding voltage of −0 mV. The data is given as the mean ± SEM of 4–5 separate experiments

all the TRPC channels, and found that only TRPC2 was expressed. Recent investigations have revealed that the phosphatase and PKA-regulated entry mechanism is, in fact, the result of activation of TRPC2 (Sukumaran et al. manuscript in preparation). Furthermore, knockdown of TRPC2 significantly hampered ATP-evoked calcium signalling [10] (see Fig. 6.1a). In addition, in these cells, patch clamp recordings revealed a significant change in conductances in TRPC2 knockdown cells, compared with control cells (Fig. 6.1b). Preliminary results also indicated that knockdown of TRPC2 significantly hampered proliferation of the cells (Löf et al. manuscript in preparation).

6.8 Perspective

The importance of TRPC2 in rodent social and sexual behaviour is undisputable. It will be of interest to see how future investigations will shed light on the processing of olfactory clues, and how this will interact with neural networks controlling e.g. behaviour. Furthermore, our findings that TRPC2 play a role in the rat thyroid gland physiology suggests that TRPC2 may have several other, yet undiscovered functions in rodents. This also opens up a multitude of possible interactions with different adaptor proteins and ion channels. Thus, TRPC2 may, in fact, play a much more important role in rodent physiology than hitherto believed.

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