TRPC5: A Startling Discovery

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Abstract: TRPC5, a protein of the canonical transient receptor potential ion channel family, is primarily found in the mammalian central nervous system. Heterologous expression of TRPC5 indicates that it forms a non-selective cation channel functioning downstream of G-protein coupled receptor (GPCR) activation, with substantial sensitivity to intracellular calcium. TRPC5 can form a related heteromeric channel with TRPC1, also controlled by GPCRs and calcium, but with unique biophysical properties. Pharmacology for TRPC5 is minimal; the only small molecules known to activate (lanthanides) and block (2-APB, SKF96365, and flufenamate) the channel are non-specific. *In vivo* and ex vivo studies have validated several roles for TRPC5 in mammals. Multiple reports implicate TRPC5 in neuronal process extension, suggesting that it could be a target for central nervous system regenerative therapies in spinal cord or brain injury. TRPC5 knockout mice exhibit reduced innate fear, making TRPC5 a candidate for the development of novel anxiolytics. While less validated, several preliminary studies suggest additional roles for TRPC5. Gonadotropin releasing hormone neurons express TRPC5 and respond to native peptide activation with a TRPC5-like current, raising the possibility for modulation of existing gonadotropin based therapies for infertility and hypogonadism. TRPC5 protein levels are elevated in a porcine model of metabolic syndrome, and may play a role in hypertension and cardiovascular disease. TRPC5 is likely expressed in trigeminal neurons, and may function in sensation. Debate continues over the involvement of TRPC5 in store-operated calcium entry, a cellular process necessary for T cell activation and the functions of many cell types.

Keywords: TRP channel, TRPC5, anxiety, genetic deletion.

INTRODUCTION

The Transient Receptor Potential (TRP) ion channel superfamily consists of 28 related proteins in mammals [1, 2]. Their functions and expression patterns are diverse with some yet to be determined; roles for TRP channels have been identified or proposed in sensory transduction, pain, and brain processing [3], calcium signaling and immune cell development [4], and regulation of cardiovascular tone [5], among others. The TRP channel proteins are divided into several families based upon homology; the canonical, or TRPC family, is most homologous to the original drosophila *trp* channel required for vision. The TRPC family itself may be further subdivided into two subfamilies, TRPC1/C4/C5 and TRPC3/C6/C7. Different proteins within subfamilies may form homomeric or heteromeric channels with one another; each combination exhibits unique biophysical properties [6-8]. Broadly, TRPC channels are activated downstream of G-protein coupled receptor (GPCR) and growth factor receptor signaling; in the case of TRPC3, C6, and C7, this occurs *via* phospholipase C (PLC) catalysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) to the effector molecule diacylglycerol (DAG), which may directly gate the channels. The native direct activators of TRPC1, C4, and C5 are unknown. There is ample evidence for multifactorial modulation of TRP channels *via* many cellular signals, including PIP_2 levels [9], pH [10], calcium [11], kinases [12], and other molecules.

TRPC5 is expressed in a number of different regions throughout the brain. In situ hybridization staining in mouse brain indicates that the highest levels of transcription occur in the hippocampus, olfactory bulb, and amygdala, with some mRNA detection in piriform cortex, some hypothalamic nuclei, and diffuse expression throughout the neocortex [13]. TRPC5 may be found in other brain regions or even other tissues at lower levels [14]. The bulk of TRPC5 studies consist of electrophysiological recordings and calcium imaging experiments utilizing heterologous expression of the channel in model mammalian cells (such as HEK or CHO). As a result of these studies, it is well established that TRPC5 is present in the plasma membrane, and mediates a non-selective cationic conductance with observable calcium permeability. Whole-cell currents of TRPC5 homomeric channels display a characteristic doubly-rectifying current-voltage relation, with a modest voltage dependence that becomes much more pronounced at higher voltages (Fig. **1A**) [15, 16]. Single channel currents indicate a substantial and non-linear single-channel conductance, suggestive of voltage-dependent divalent effects on permeation through the channel's pore, such as flickery block by calcium [17, 18]. When TRPC5 is co-expressed with TRPC1, a unique channel is formed. The heteromeric channel's whole-cell current-voltage relation is strongly outwardly rectifying with a minimal inward conductance, accompanied by a dramatic reduction in single-channel conductance when compared to the homomeric channel (Fig. **1B**) [7].

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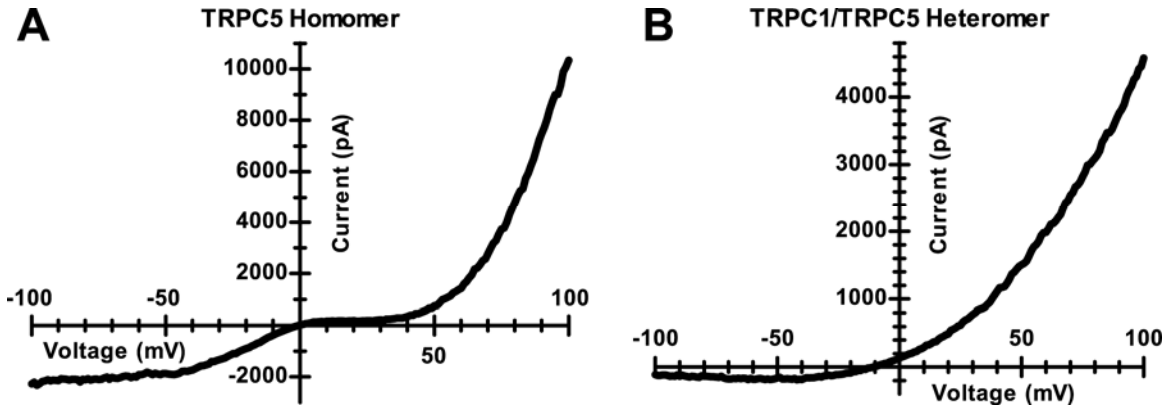

Fig. (1). TRPC5 current-voltage relations. (**A**) TRPC5 homomeric channels produce an unusual doubly rectifying current-voltage relation, reversing slightly below 0 mV. (**B**) TRPC1/TRPC5 heteromeric channels produce a more outwardly rectifying current-voltage relation with reduced inward current, and reverse slightly below 0 mV.

The differences between the two channels suggest that TRPC1/C5 is likely to be a functionally distinct channel from the TRPC5 homomeric channel, but may share some pharmacology. There is evidence of both homomeric and heteromeric channels in brain. TRPC1/C5 heteromeric channels are expressed in the cell bodies and processes of cultured hippocampal neurons but are excluded from growth cones, while homomeric channels are found throughout the cell including growth cones [19]. Both homomeric TRPC5 and heteromeric TRPC1/C5 channels may be activated downstream of GPCRs that couple to G-proteins containing the $G\alpha_{q/11}$ subunits. Both channels are also substantially modulated by the intracellular calcium concentration, so care should be taken to control intracellular calcium while studying these channels [20, 21]. Their calcium sensitivity may indicate a propensity for functioning downstream of intracellular calcium signaling cascades. Indeed, TRPC5 contains multiple calmodulin and other calcium binding protein interaction sites, and is subject to regulation by Protein Kinase C [12]. TRPC1 and TRPC5 pharmacology is limited, but both channels are potentiated by micromolar concentrations of trivalent lanthanides (lanthanum and gadolinium); the site of this interaction has been mapped to an extracellular region near the mouth of TRPC5's putative pore [22]. TRPC5 is blocked by the non-selective channel blockers 2-APB, SKF96365, and flufenamate [15, 23].

Many functional roles for TRPC5 have been proposed, including neurite outgrowth in cultured neurons, innate fear responses in TRPC5 knockout mice, gonadotropin releasing hormone (GnRH) secretion from GnRH neuron-derived cell lines, and possible roles in metabolic syndrome, trigeminal neurons, and store-operated calcium entry. These putative functions make TRPC5 a good candidate for drug development in multiple therapeutic areas.

IN VIVO AND *EX VIVO* VALIDATED ROLES FOR TRPC5 IN MAMMALS

Neurons are highly polarized cells, with specialized processes designed for computation and communication. Dendrites typically receive information from other neurons and process multiple incoming signals, while axons transmit information to targets of their originating neuron. Axon pathfinding (extension or outgrowth) from neuron cell bodies to other neuronal and tissue targets is critical for proper development and functioning of the nervous system; dendrite outgrowth into receptive fields is similarly important. At the tip of extending processes are specialized structures termed growth cones; these cones respond to internal and environmental cues (secreted and membrane bound factors) to direct the growth of the process [24]. Inhibition of neuron process growth is thought to contribute to the inability of the adult nervous system to regenerate and repair damage suffered from insults such as injury and stroke [25]. Greka *et al*. proposed a negative regulatory role for TRPC5 in neuronal process extension [26]. Immunostaining indicated the presence of TRPC5 in growth cones of cultured hippocampal neurons, and TRPC5 homomeric channel-like single channel events could be recorded from those growth cones. Expression of a TRPC5 dominant-negative protein, which presumably inhibited the channel's activity, led to increased neurite length in these neurons.

More recent reports, while affirming a role for TRPC5 in neuronal process growth, conflict on the sign of this interaction, suggesting that TRPC5 activation may contribute positively to neurite extension. In one study, RNAi directed against TRPC5 reduced ganglioside-induced calcium influx and neurite outgrowth in a neuronal-derived cell line [27]. In another, CaMKI calcium-dependent axon outgrowth initiation in cultured hippocampal neurons was diminished by RNAi against TRPC5, but increased by application of lanthanum, a TRPC5 potentiator [28]. Both studies demonstrated a clear correlation between increased calcium influx and the initiation of neurite formation; whether neurite initiation and extension are dissimilar enough for TRPC5 to function inversely from one to other remains to be elucidated. Another intriguing possibility is that TRPC5 might function differently in axons as opposed to dendrites. Perhaps TRPC5 could be a novel target for neuronal regenerative therapies. There is also a hotly debated new theory that depression may be the result of decreased neurogenesis, or birth of new neurons, in certain regions of the brain [29]. TRPC5 might modify the growth of axons and dendrites in newly differentiated neurons, allowing them to make proper connections. Whether activation or blockade of TRPC5 would prove more effective in stimulating proper neurite growth is unclear. The TRPC5 knockout mouse would be expected to exhibit abnormal connectivity in the

brain, but it is impossible to predict the functions or regions that might prove most susceptible to the loss of TRPC5.

Fortunately, the initial characterization of the TRPC5 knockout mouse was recently published; TRPC5 -/- mice displayed a reduction in innate fear responses to loud or painful stimuli (that is, startle responses), but conditioned fear responses remained normal [30]. From preliminary tests, the TRPC5 -/- mice seem to possess no fear-learning, acute sensory or attention deficits, but may be less anxious at baseline. Acutely isolated neurons from the lateral amygdala of TRPC5 -/- mice lacked a cholecystokinin-2 (CCK-2) induced post-synaptic current, attributed to TRPC5 homomeric channels, that was present in wild-type mice. Metabotropic glutamate receptor (mGluR) and CCK2-dependent firing rates were also reduced in amygdaloid neurons from TRPC5 -/- mice. Long-term potentiation of synaptic strength, an electrophysiological model of learning, was unaffected at these TRPC5 -/- amygdala synapses, consistent with the lack of any fear-dependent learning deficits. It should be noted that this was a global knockout; it is likely but not certain that the reduction in innate fear derived from a lack of functional TRPC5 responses, rather than some developmental change resulting from the absence of TRPC5. The knockout data suggest TRPC5 blockade as a novel target for the reduction of anxiety; specific antagonists are strongly desired to continue these studies.

PRELIMINARY STUDIES OF TRPC5

Kisspeptins and their cognate G-protein coupled receptor, GPR54, are critical regulators of entry into puberty and sexual maturation in humans and mice [31]. GPR54 activation by kisspeptins causes a depolarization that results in production of gonadotropin releasing hormone (GnRH) from GnRH neurons. The gonadotropin system is commonly targeted for pharmacological intervention during treatment of infertility and hypogonadism. Several groups have reported expression of TRPC5 in GnRH neuron-derived cell lines [32, 33]. The conductance underlying the GPR54-dependent depolarization bears some resemblance to TRPC5 homomeric channels. Although further research is needed in this area, modulation of TRPC5 might be a method for modifying or fine-tuning gonadotropin related fertility treatments for both men and women, and may offer another target for treatment of hypogonadism.

Metabolic syndrome is indicative of increased cardiovascular disease risk, and typically presents with a combination of hypertension, insulin resistance, obesity, and dislipidemia. Hu *et al*. have recently proposed a pathological role for TRPC5 in metabolic syndrome; they observed elevated TRPC1, C5 and C6 expression in pigs with metabolic syndrome [34]. Additionally, calcium signalling in adrenal medullary cells, a presumable site of primary pathology in metabolic syndrome, was not inhibited by lanthanum. Although lanthanum blocks most calcium permeable channels, it potentiates TRPC5. Although minimal functional data support a role for TRPC5 in metabolic syndrome, it is an interesting area for further development, with TRPC5 blockers potentially bringing some relief to sufferers of the disease.

Trigeminal neurons are the first-order sensory receptors for the face. They process tactile, proprioceptive, temperature, and nociceptive stimuli and transmit them to the brain. Gomis *et al*. have proposed that TRPC5 may act as a mediator of mechanical stimuli in trigeminal neurons [35]. TRPC5 expression was prominent in cultured trigeminal neurons. TRPC5 also responded to mechanical disturbances of the cell's membrane and osmotic pressure gradients when studied in a heterologous expression system; however, the effect was calcium dependent, bringing its specificity into question given the sensitivity of TRPC5 to calcium. Nevertheless, the presence of TRPC5 in trigeminal neurons provides an intriguing possibility for TRPC5 function in sensation.

The earliest studies of TRPC5 proposed a role for the channel in capacitative, or store-operated, calcium entry; this remains controversial. When calcium is depleted from the endoplasmic reticulum 'store', a plasma membrane-delimited calcium influx pathway is activated. This influx pathway is presumably an ion channel; such an ion channel would therefore be described as store-operated. The most widely studied model of store-operated calcium entry is the calcium release activated calcium current, or I_{CRAC}; this highly selective calcium current is required for T-lymphocyte activation. I_{CRAC} initiates upon depletion of calcium from the endoplasmic reticulum following activation of the T cell receptor complex, and is necessary for both continued calcium signaling and replenishment of the endoplasmic reticular calcium store [36]. However, store-operated calcium entry occurs in many cell types with differing biophysical features, downstream of signals that cause intracellular calcium release. There are early reports both confirming and denying the activation of TRPC5 channels in response to the emptying of endoplasmic reticulum calcium stores [6, 15, 16]. Following the recent discovery of two critical protein components of I_{CRAC}, the endoplasmic reticulum calcium sensor STIM and the calcium channel ORAI [37], some groups have reported biochemical interactions between TRPC5 and these proteins [38]. Other groups have disputed this claim, and demonstrated no reduction of either TRPC5 homomeric channel current in the absence of STIM or ORAI, nor any loss of store-operated calcium currents in the absence of TRPC5 (achieved *via* RNAi knockdown). At the moment, the evidence against a role for TRPC5 in store-operated calcium influx seems more compelling.

CONCLUSION

Although substantial further work remains to validate TRPC5 as a target in a number of therapeutic areas, there are some indications that may warrant directed drug discovery at this time. With a specific small molecule blocker in hand, the role of TRPC5 in anxiety is intriguing and can be studied using established protocols and animal models. The same is true of metabolic syndrome especially when hypertension is considered as the assay. With blockers and good models of mammalian fertility, the function of TRPC5 in the gonadotropin pathway could be probed with reasonable accuracy. Adding TRPC5 modulators to the constellation of treatments for CNS regeneration places this indication some distance from any marketable product, but could produce exciting discoveries with patience. Other indications for TRPC5 should probably wait for further validation from the basic research community.

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