GENOMIC AND NON-GENOMIC REGULATION OF TRPM8 COLD RECEPTOR BY ANDROGENS

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Introduction

The member of melastatin subfamily of transient receptor potential (TRP) channel family, TRPM8, which is activated by cooling temperatures (<28 °C, with maximal activation around 18 °C) or by the chemical imitators of cooling sensation such as menthol, icilin, and eucalyptol is primarily expressed in sensory neurons where it functions as a cold receptor (McKemy *et al.*, 2002). Recently, using three independent groups of TRPM8-knockout mice have established that TRPM8 is indeed the principal detector of environmental cold (Bautista *et al.*, 2007; Colburn *et al.*, 2007; Dhaka *et al.*, 2007*)*. TRPM8-deficient mice have severe deficits in avoiding cold temperatures and in paw withdrawal responses to acetone evaporative cold stimuli and to cold-inducing icilin application, suggesting that TRPM8 activation also mediates generation of unpleasant signals sent to the brain.

However, TRPM8 is present not only in sensory neurons. Outside the nervous system, it is most abundantly expressed in the prostate, the tissue which is not involved in temperature-dependent functions. In fact, TRPM8 was first cloned from human prostate as a prostate-specific gene (Tsavaler *et al.*, 2001) even before its role in the cold sensation was established. Moreover, whilst remaining at moderate levels in normal prostate, TRPM8 expression strongly increases in prostate cancer. Other non-prostatic primary human tumors of breast, colon, lung, and skin also become highly enriched in TRPM8, whereas in the corresponding normal tissues it is virtually undetectable (Tsavaler *et al.*, 2001). All this suggests that aside from cold sensing demonstrated so far for sensory neurons, TRPM8 may have other important functions as well as modes of activation and regulation, especially in the prostate, and during carcinogenesis.

Genomic regulation of TRPM8 expression by androgens in the prostate

Interestingly, although many studies have characterized TRPM8 as a plasma membrane (PM) cationic channel involved in cold-evoked excitation in sensory neurons, the classical, neuronal-like biophysical features of plasma membrane TRPM8 ($_{PM}$TRPM8) have not yet been firmly established for prostate cells. Indeed, our experiments in the human lymph node carcinoma of the prostate (LNCaP) cell line, in which TRPM8 is highly expressed, have shown that functional TRPM8 channel is exclusively localized in the membrane of endoplasmic reticulum (ER), where it acts as Ca^{2+} release channel ($_{ER}$TRPM8) responsible for the cold-, menthol- or icilin-evoked Ca^{2+} store depletion and concomitant activation of store-operated Ca^{2+} entry (SOCE), whereas plasmalemmal TRPM8 (i.e., $_{PM}$TRPM8) is not functional in these cells (Thebault *et al.*, 2005). However, our further studies in primary cultures of human normal prostate (NP), benign prostate hyperplasia (BPH), and prostate cancer (PCa) epithelial cells (from tissue specimens obtained

during resection surgeries performed on clinical indications on patients who gave informed consent) have established a more complex picture of TRPM8 expression and function (Bidaux *et al.*, 2007). By combining electrophysiology, Ca^{2+} imaging, molecular and cell biology techniques we have discovered that only highly differentiated apical/luminal epithelial cells, characterized by the expression of apical phenotype markers, cytokeratins 8 and 18 (CK8 and CK18), rather than the basal phenotype markers CK5 and CK14 showed the presence of $_{PM}$TRPM8. Moreover, the expression of TRPM8 and cold/menthol-activated membrane current due to its PM localization and function of TRPM8 increased in PCa cells vs. NP and BPH cells. This also was in direct correlation with the expression of androgen receptor (AR): CK5/CK14-positive basal cells lacked AR and $_{PM}$TRPM8-like response, whilst CK8/CK18-positive apical/luminal cells expressed AR and showed robust $_{PM}$TRPM8-like response. At the same time functional responses associated with ER localization of TRPM8 were little dependent on differentiation status of epithelial cells and AR expression. The conclusion on PM localization and function of TRPM8 channel only in the fully differentiated, CK8/CK18-positive apical/luminal phenotype was also confirmed on epithelial cells acutely isolated from normal rat prostate.

In the pioneering paper by Tsavaler *et al.* (2001), the expression of two different TRPM8 transcripts of 6.2 kb and 5.2 kb most likely arising from *trpm8* gene splicing was found in the human prostate, although only the longer one has been cloned to date. Thus, we hypothesized that the expression of a N-terminus truncated TRPM8 splice variant could be responsible for the AR-independent $_{ER}$-TRPM8 activity in all prostate epithelial cell phenotypes, whereas full-size TRPM8 variant, whose expression is regulated by AR is responsible for the classical $_{PM}$TRPM8-mediated response in the form of cold/menthol-activated membrane current. Previous studies have established several putative androgen response elements in *trpm8* gene, however, the existence of an alternative promoter, which may make the $_{ER}$TRPM8 isoform less sensitive to androgens than $_{PM}$TRPM8, cannot be excluded.

Non-genomic regulation of TRPM8 in sensory neurons

Given that a number of sensory modalities including cold and warmth perception are affected by changes of the individual hormonal status (Doeland *et al.*, 1989; Harju, 2002; Potkanowicz *et al.*, 2003), which in turn is a variable of gender, age, seasonal, and dietary conditions, physical and psychosocial state, one can expect that sex steroid hormones regulate cold sensitivity by altering TRPM8 expression and/or function also in sensory neurons. Consistent with this prediction, our behavioral tests on male and female mice using cold/warm plate preference test have shown that male animals exhibit reduced sensitivity to the innocuous cold temperatures in the range of 15-25 °C compared to females and that this

is associated with the reduced density of TRPM8-mediated membrane current (I_{TRPM8}) and shifted voltage-dependence of its activation to more depolarized potentials in male's vs. female's DRG neurons (Kondratskyi *et al.*, 2009). Some behavioral tests conducted on sham-operated and orchidectomized (OX) male rats with and without systemic siRNA-mediated TRPM8 silencing allowed us to conclude that *in vivo* cold sensitivity in the innocuous range of cooling temperatures is decreased by higher circulating testosterone levels and that this decrease is due to androgenic control of TRPM8 cold receptor. Moreover, the fact that endogenous TRPM8 mRNA levels were not altered in the DRGs from OX vs. sham-operated male rats suggested to us that this control is not associated with TRPM8 gene expression, but is rather due to the inhibitory action of androgens on TRPM8 channel function either directly or *via* the putative non-conventional surface steroid receptor(s).

Using the model system of heterologously expressed TRPM8 in HEK-293 cells (which lack endogenous classical AR) as well as native DRG neurons we have shown that TRPM8 is the subject of inhibitory action of testosterone, which in physiologically relevant concentrations (10-100 nM) suppresses cold-, menthol- or icilin-activated I_{TRPM8} in both cell types as well as menthol-evoked action potential firing in DRG neurons. Experiments aimed at determining the mechanism of testosterone action revealed that it involves putative, non-conventional surface testosterone receptor coupled via pertussis toxin-(PTX)-sensitive Gi protein to the adenylate cyclase (AC) — cAMP — protein kinase A (PKA) signaling pathway. Since engagement of Gi inhibits catalytic activity of AC and reduces cAMP levels, testosterone-mediated suppression of TRPM8 could be the consequence of decreased basal PKA-dependent phosphorylation of TRPM8 channels or accessory protein(s) impairing TRPM8 activity.

Our data are consistent with the notion that elevated plasma levels of testosterone both in males and females, which usually accompany mating behaviours, physical activity, stress, aggression by desensitizing TRPM8 will help to diminish the impact of environmental cold as a factor which may impede taking necessary actions.

Conclusions

TRPM8 is the subject of genomic and non-genomic regulation by androgens. The first mode of regulation is more specific to non-neural, particularly prostate epithelial cells. It involves differential, AR-dependent expression of two, ER- and PM-localized, TRPM8 splice variants. The expression of $_{ER}$TRPM8 is little AR- and epithelial cell phenotype-dependent, whereas the expression of $_{PM}$TRPM8 is strongly dependent on AR, is determined by the differentiation status of prostate epithelial cells peaking in the fully differentiated apical/luminal cell phenotype and is further enhanced upon transition to the prostate cancer. This makes $_{PM}$TRPM8 a viable marker for the prostate cancer staging. In addition to the genomic regulation by androgens of TRPM8 expression detected in the non-neural

cells, TRPM8 in the peripheral nervous system is also the subject of the short-term, non-genomic inhibition by testosterone which involves putative PM testosterone receptor coupled to the Gi/AC/cAMP/PKA pathway. Depending on the levels of circulating androgens, this type of regulation may notably impact the perception of innocuous cold on the whole organism level.

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