Expression of TRPM8 in the prostate of rams and wethers

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INTRODUCTION

The Transient Receptor Potential Melastatin 8 (TRPM8) channel is important for the somatosensory detection of cold. Agonists of this channel include menthol and icilin. Androgens, specifically testosterone, are known to regulate TRPM8 function and expression (Liu et al., 2016). In prostate cancer cells, TRPM8 expression could only be detected in cells that were positive for the androgen receptor, and were not found on cells that did not produce the androgen receptor (Bai et al., 2010). In healthy prostate epithelial cells, the expression of TRPM8 is rather low compared with carcinoma cells of the prostate where TRPM8 expression is increased and highly dependent on androgen concentrations (Kulkarni, 2009). These channels are also known to be important for bladder function and to a larger extent male fertility (Liu et al., 2016). In mice, the TRPM8 channels have been found in the head region of sperm cells and enable the sperm cell to detect temperature changes, as well as a possible role for these channels in initiating the acrosome reaction (Martínez-López et al., 2011). Given that TRPM8 channels bind testosterone and are dependent on concentrations of testosterone, ram prostate should have increased expression of TRPM8 compared with a wether that would have low concentrations of circulating testosterone. The goal of this research was to quantify TRPM8 expression in the prostate of rams compared with wethers and determine if the observed differences may be affected by concentrations of testosterone, which may contribute to fertility in the rams.

MATERIALS AND METHODS

All animal protocols were approved by the University of Wyoming Institutional Animal Care and Use Committee.

Rams (n = 6) and wethers (n = 6) were anesthetized with a ketamine–rompun mixture (100 mg/mL 10:1 ratio) injected intravenously through the jugular vein. Following anesthesia animals were exsanguinated and tissues were collected. Ewe ovaries and ram testes were collected for relative comparisons. This proceedings paper will focus on expression of TRPM8 in the prostate and testes.

Frozen tissue (100 mg) was used for mRNA isolation first using Tri Reagent, and then further purified using the Qiagen Rneasy mini kit. Real-time semi-quantitative RT–PCR was performed using the Iscript cDNA synthesis kit and the SYBR Green procedures of BIORAD; data were quantified using the 2^{-ddCT} method of Livak and Schmittgen.

Protein isolation and western blots were performed using previously reported procedures of Johnson et al. (1998). Briefly, 100 mg of tissue was homogenized using a mechanical homogenizer; the resulting homogenate was centrifuged to remove debris, quantified by spectrophotometry at 280 nm and 100 µg lysate was aliquoted, boiled and applied to each lane of a 12% denaturing polyacrylamide gel. Following electrophoresis, gels were transferred to 0.2 µM nitrocellulose in Towbin buffer. Membranes were blocked in 5% nonfat dry milk and detected using rabbit anti-TRPM8

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antibody (1:1000; Lifespan Biosciences) followed by HRP conjugated second antibody (Jackson Labs; 1:10,000) and chemiluminescent substrate (Thermo fisher). Intensity of bands on x-ray film were quantified using UNSCANIT (Silk Scientific).

Data were analyzed using a nonparametric analysis (Kruskal–Wallis SAS version 9.3).

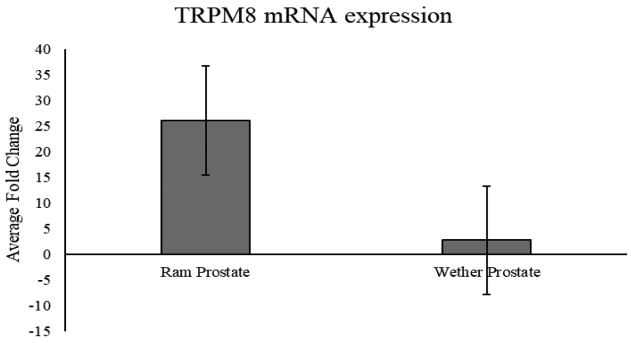

Figure 1. TRPM8 mRNA expression in the prostate of rams compared with wethers was different (P = 0.01). Rams had higher average fold change in the prostate compared with wethers.

RESULTS AND DISCUSSION

Results from real-time semi-quantitative RT-PCR showed that rams had increased (P = 0.01) TRPM8 mRNA expression compared with wethers (Figure 1). In a relative comparison of gonadal tissues and prostate, the ram testes had the greatest mRNA expression, followed by the ovaries from the ewes, then the ram prostate, and the wether prostate (Figure 2). The wethers had the least amount of mRNA expression compared with the other tissues. The results obtained for protein levels using western blot differed from the expression of mRNA in the same tissues. There was no difference (P>0.1) in the expression of protein in the prostate between rams and wethers (Figure 3). The order of the other tissues was similar to that seen in the mRNA expression; with testes having the greatest expression, followed by the ovaries, and the prostate (Figure 4). Protein concentrations were determined by Western blot (Figure 5). Although

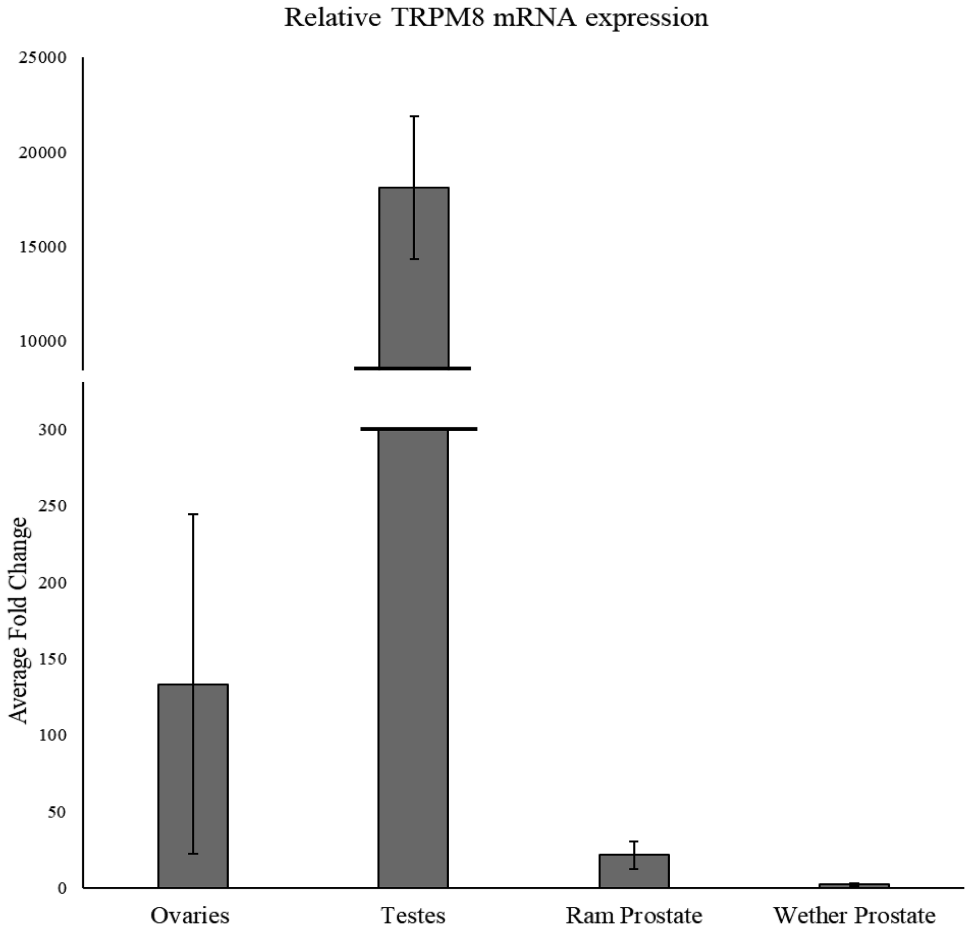

Figure 2. Relative TRPM8 mRNA expression in testes, ovaries, and prostate. Testes have the highest relative TRPM8 mRNA expression, followed by the ovaries, ram prostate, and the wether prostate.

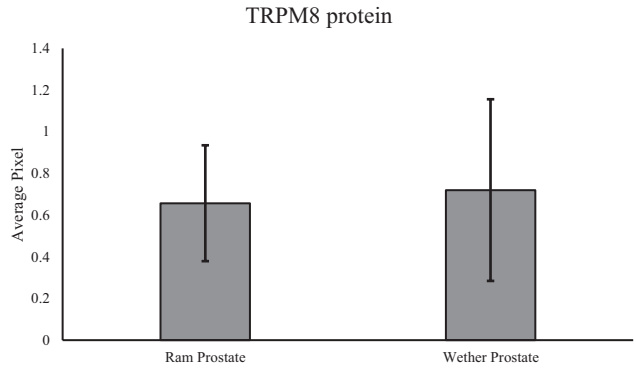

Figure 3. TRPM8 protein in the prostate of rams compared with wethers was not different ($P > 0.1$). There was no difference in the average pixel number between rams and wethers in the prostate.

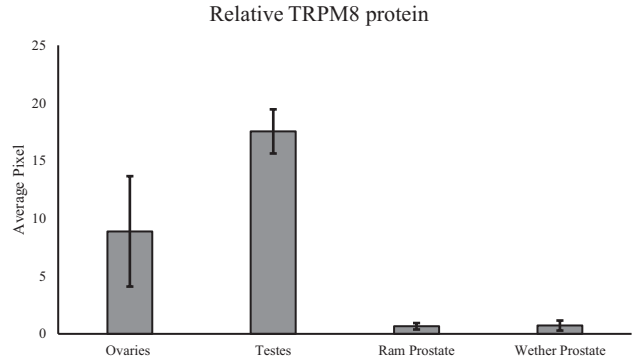

Figure 4. Relative TRPM8 protein in testes, ovaries, and prostate. Testes have the highest relative TRPM8 protein, followed by the ovaries, wether prostate, and the ram prostate.

Figure 5. X-ray film showing protein bands which were quantified for TRPM8 (top) and β-actin bands (bottom).

the protein and mRNA expression were not parallel, this could be due to the variability in mRNA expression.

Greater mRNA expression was observed in the prostate of rams compared with wethers. Reduced mRNA expression could be a result of downregulation of TRPM8 in wethers who do not have high/sufficient serum concentrations of testosterone, an activator of these channels. In the prostate TRPM8 is localized in the epithelial cells, and could be an important factor in calcium homeostasis of the cells (Kulkarni, 2009). A majority of TRPM8 research in the prostate has focused on cancer research. In prostate carcinoma cells the expression of TRPM8 is up regulated compared with normal cells. Prostate carcinoma cells are sensitive to androgen concentrations and require high androgen concentrations for growth and cell survival (Kulkarni, 2009; Liu et al., 2016). Demirkhanyan et al., 2018 hypothesized that TRPM8 channels might directly influence sexual behavior of animals since these channels are influenced by testosterone dependent signaling (Demirkhanyan et al., 2018). From the results presented in this paper TRPM8 expression may influence functionality and efficiency of the male reproductive tract.

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