

Quantitative comparison of TRPM8 positively stained neurons in the hypothalamus and amygdala of rams categorized behaviorally as low or high sexual performers

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INTRODUCTION

The transient receptor potential melastatin 8 (TRPM8) is important for the somatosensory detection of cold, with menthol and icilin being known activators of the TRPM8 channels. Recently identified as a testosterone receptor (Asuthkar et al., 2015a, 2015b), TRPM8 has also been implicated in prostate cancer cell survival (Zhang and Barritt, 2004). Unpublished observations in TRPM8 knockout mice suggested a role for these channels in the expression of male behavior. Since TRPM8 is a known testosterone receptor, it is plausible that these channels would have a role in the expression of male sexual behavior. The goal of this research was to quantify TRPM8 receptors in regions of the brain known to be important for the expression of sexual behavior or endocrine function.

MATERIALS AND METHODS

Ram sexual behavior was determined by service capacity test where rams were individually exposed to ewes ($n = 2-3$) in estrus for 20 minutes. Rams that mounted ewes within 10 min of the test, achieved ≥ 6 ejaculations in 20 min, and exclusively mounted females were considered high performing (HP) rams. Low performing (LP) rams were those that failed to exhibit sexual interest toward either male or female stimulus animals,

showed a long latency (>10 min) to mount, and only achieved ≤ 3 ejaculations during the test.

Rams were isolated from ewes and housed individually while maintaining visual and olfactory contact. On the day of tissue collection, rams were exposed to urine from ewes in estrus for 1 hr. Following exposure, rams were anesthetized and exsanguinated; brains were perfused using paraformaldehyde and collected.

Brains were blocked using surface landmarks and tissue sections of the amygdala and ventral medial hypothalamus were cut rostral to caudal. Tissues were stained using an immunohistochemistry staining protocol, with anti-TRPM8 polyclonal antibody (Lifespan Biosciences, Seattle, WA) as the primary antibody. Stained tissue was observed at $10\times$ with digital photographs taken for quantification. Data were analyzed using the non-parametric analysis of SAS (Kruskal–Wallis test; SAS version 9.3). All animal procedures were approved by the University of Wyoming animal care and use committee.

RESULTS AND DISCUSSION

Differences in the number of TRPM8-positive cells in the amygdala or ventral medial hypothalamus were not noted ($P > 0.3$) among HP and LP rams. Although staining was not noted in the endocrine hypothalamus, robust staining was noted in the dorsal-lateral hypothalamus. It is possible that these positively stained cells could be interacting downstream with endocrine cells. The lateral hypothalamus is known to affect a

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variety of motivated behaviors, such as food intake, water intake, salt intake, and even to some extent sexual behavior (Hurley and Johnson, 2014). These motivated behavior pathways, when acted upon, promote a goal-driven behavior and an encouraged locomotor behavior that would suggest a state of psychological arousal (Hurley and Johnson, 2014). Neurons located in the central nucleus of the amygdala are strongly associated with a sense of fear in humans (Pitts et al., 2009). In sheep, it is likely that these neurons are attributing to an amplified sense of awareness, leading to increased arousal. The TRPM8 channels have been shown to be regulated by testosterone; where high concentrations of testosterone will decrease the expression of these channels (Asuthkar et al., 2015a, 2015b). Downregulation of these receptors would lead to greater fear tolerance in animals with increased levels of testosterone. The lateral hypothalamus and the amygdala appear to contribute toward an elevated sense of arousal in the animal, and could explain why positively stained neurons were observed in these areas. The mesolimbic dopamine system includes neurons that run through the lateral hypothalamus and are associated with motivating and rewarding behaviors (Hurley and Johnson, 2014). Hypocretin, also known as orexin, is a neuropeptide sent via distal projections to diverse areas of the brain from a neurotransmitter system relatively confined to specific areas including the dorsal medial and the lateral hypothalamus (Hurley and Johnson, 2014). Orexins in the lateral hypothalamus are known to be activated by estradiol in male mice, produced from a local conversion of testosterone by aromatase (Muschamp et al., 2007). Activation of

orexins by estradiol then transmits signals to the ventral tegmental area and work within the dopamine neural pathway (Muschamp et al., 2007). In the ram, expression of TRPM8 channel in the amygdala and the hypothalamus may not be important for the expression of sexual behavior precisely, but may be important for the integration of sensory signals and influence the biological significance of those stimuli.

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