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Reproductive and metabolic responses of desert adapted common spiny male mice (*Acomys cahirinus*) to vasopressin treatment

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ABSTRACT

Sufficient amounts of water and food are important cues for reproduction in an unpredictable environment. We previously demonstrated that increased osmolarity levels, or exogenous vasopressin (VP) treatment halt reproduction of desert adapted golden spiny mice *Acomys russatus*. In this research we studied gonad regulation by VP and food restriction (FR) in desert adapted common spiny mouse (*A. cahirinus*) males, kept under two different photoperiod regimes—short (SD—8L:16D) and long (LD—16L:8D) days. Mice were treated with VP, FR, and VP + FR for three weeks. Response was assessed from changes in relative testis mass, serum testosterone levels and mRNA receptor gene expression of VP, aldosterone and leptin in treated groups, compared with their controls. SD-acclimation increased testosterone levels, VP treatment decreased expression of aldosterone mRNA receptor in the testes of SD-acclimated males. FR under SD-conditions resulted in testosterone decrease and elevation of VP- receptor gene expression in testes. Aldosterone receptor mRNA expression was also detected in WAT. These results support the idea that water and food availability in the habitat may be used as signals for activating the reproductive system through direct effects of VP, aldosterone and leptin on the testes or through WAT by indirect effects.

1. Introduction

Changes in day length (photoperiod) are the main environmental cue for physiological seasonal changes and therefore, they can serve as an important signal for the coming breeding season for mammals inhabiting predictable ecosystems in temperate zones (Bronson, 2004, 2009). In unpredictable ecosystems, like deserts photoperiod changes are not a sufficient signal to predict the optimal time of breeding. In order to insure survival in such an ecosystem it is crucial to adjust reproduction and delivery to recourses availability. Therefore, integration of various environmental cues is an important adaptation for the optimal time of breeding and for survival in xeric environments. (Schwimmer and Haim, 2009). In regards we asked the following question: What are the environmental cues used by xeric adapted small mammals for optimal time of breeding in an unpredictable environment?

An osmotic stress was noted as an effective internal signal for reducing reproductive activity in the golden spiny mice *Acomys russatus* (Shanas and Haim, 2004) and in a desert adapted population of common spiny mice *Acomys cahirinus* (Bukovetzky et al., 2012). Reduced water availability was found to decrease epididymal and seminal vesicle masses in the Californian mouse *(Peromyscus californicus)*, which is a temperate zone rodent which differs from most other species, it breeds under short days as water availability in the environment affects reproductive function (Nelson et al., 1995).

Vasopressin (VP), a hormone involved in water balance and osmoregulation is produced by the hypothalamus and released by the neuro-pituitary. VP secretion levels increase in response to elevated plasma osmolarity, representing water shortage. Therefore, increased water re-absorption at the nephrodistal tube and collecting tubes (Schmidt-Nielsen and Schmidt-Nielsen, 1952) is important under osmotic stress. Yet, several studies link VP to reproduction. VP receptors are found in the reproductive tract (Maggi et al., 1988; Lolait et al., 1995). VP injection was found to increase the amplitude and frequency of epididymal contraction in mice (Miller et al., 2006). Results from the extreme desert rock dwelling species *A. russatus* revealed that VP treatment, similar to salinity increase of water source, inhibited reproduction in females (Shanas and Haim, 2004) and in males (Wube et al., 2008).

Aldosterone is also known as an osmoregulatory hormone that plays an important role in the regulation of sodium excretion through the renin–angiotensin–aldosterone pathway. Its activation results in extremely potent vasoconstriction, affecting also the genital tract, thus it may serve as a candidate of controlling reproduction under draught conditions. Angiotensin II receptors were discovered in

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human reproductive tract, males and females, suggesting a multiplicity of roles that are unrelated to aldosterone and angiotensin II primary osmoregulatory functions (Hassan et al., 2006). Changes in VP and aldosterone hormone levels on metabolism and reproduction and receptor expression of VP on gonads make them candidates for transferring environmental signals for water availability to the reproductive system of desert adapted rodents.

The amount of body fat influences fertility, indicating a link between white adipose tissue (WAT) amounts and the reproduction system (Frisch, 1990; Zhao and Wang, 2006). For rodents as small mammals, changes in the quantity and quality of food have major effects on reproduction (Bronson, 1989). Food restriction can result in delay of the onset of sexual maturity, suppression of the estrous cycle, uterus atrophy, and absorption of embryos during gestation, decrease in milk production and changes in plasma reproductive hormone concentrations (Nelson and Demas, 1996; Long et al., 1999; Blache et al., 2000; Angel-Meza et al., 2001; Kusina et al., 2001). However, food supplementation can improve reproductive capacity in animals promoting earlier onset of sexual maturity as estrous cycle activation (Colares, 1997; Long et al., 1999; Kusina et al., 2001). Leptin administration prevented the disruptive effects of acute food deprivation on reproductive function in cycling and lactating rat females suggesting that leptin levels contribute to the regulation of reproductive function (Abizaid et al., 2004).

Based on previous results describing the importance of water and food availability for successful breeding in an unpredictable ecosystem, we tested the following hypotheses: If water and food availability affect breeding through the involvement of VP, aldosterone and leptin hormones than 1). Modifications in these hormone receptors gene expression will be noted in the gonads as a response to different environmental conditions representing water availability in the habitat. 2). A correlation between gonads status and leptin plasma levels is expected.

To test these hypotheses, we examined the effects of exogenous VP, food restriction and a combination of food restriction with VP-treatment on the reproductive activity of *A. cahirinus* males from a desert adapted population, acclimated to short and long photoperiods.

2. Materials and methods

2.1. Animals

Four months old males with the similar initial body mass (W_b) (35 ± 4 g) from a desert adapted population of *A. cahirinus* were recruited from a breeding colony maintained at Oranim campus, University of Haifa. Mice in the colony are descendants of individuals originally captured on the western shores of the Dead Sea, Israel. In the colony, mice are kept at an ambient temperature (T_a) of 26 ± 2 °C under a photoperiod regime of 12L:12D.

2.2. Photoperiod and treatment

Twenty-four *A. cahirinus* males were acclimated to short day (SD—8L:16D) while twenty six males were acclimated to long day (LD—16L:8D) inside a cabinet (158 × 77 × 74 cm) (Meditest 600/1300, Austria) for three weeks (Shanas and Haim, 2004). Lights were on between 07:00 and 15:00 h for SD; 07:00 and 23:00 h for LD. Mice were kept in separate transparent polycarbonate cages (35 × 25 × 15 cm). Food (rat pellets, Koffolk, Israel) and 2% agar gel cubes (20 g of dry agar dissolved in 1000 mL of deionized water) as a source of water were provided *ad libitum*. Salinity concentration in the agar cubes was 0.9% NaCl for all studied groups. Mice were weighed to the nearest 0.1 g using a digital balance (Sartorius 5500, Germany). Cabinet temperature was set at their lower critical point 28 ± 1 °C (Weissenberg and Shkolnik, 1994). All experimental procedures were approved by the ethical committee, University of Haifa.

After three weeks of acclimation to SD or LD, mice from both photoperiods' acclimated groups were divided into sub-groups for the following treatments:

1) Control group—*i.p.* administrations of sterile saline solution (0.9% NaCl), $n = 6$ for SD and $n = 6$ for LD, acclimated mice.
2) VP group—mice were *i.p.* injected with VP solution (50 μg/kg W_b, Sigma) three times a week (Shanas and Haim, 2004; Wube et al., 2008), $n = 6$ for SD and $n = 6$ for LD, acclimated mice.
3) Food restricted (FR) group—mice were offered 75% of their 100% food intake, calculated from their average food intake at the last week of photoperiod acclimation, in addition sterile saline solution (0.9% NaCl) was administrated *i.p.*, $n = 6$ for SD and $n = 7$ for LD, acclimated mice.
4) VP + FR group—mice were exposed to combined treatment of VP and FR ($n = 7$), $n = 6$ for SD and $n = 7$ for LD, acclimated mice.

2.3. Sacrificing the animals

At the end of each experiment mice were anesthetized by injecting a cocktail of Ketamin (10 mg/kg) and Rampoone (100 mg/kg) followed by decapitation. All mice were sacrificed between 10:00–12:00 h. (For both photoperiod groups starting three hours after lights were on). Blood samples were taken for analyzing serum testosterone levels. WAT and testes were removed, weighed and frozen at −20 °C until further analysis was carried out. The testis mass were calculated as percent of W_b.

2.4. Testosterone and leptin levels detection

Serum testosterone levels were measured using ELISA (Cayman chemical, USA) and optical density (OD) was obtained by an ELISA reader (Power Wave XS, BioTek., Gen 5) at 410 nm wavelength. Serum Leptin levels were measured by Quantikine Mouse Leptin Immunoassay (R&D Systems Inc., USA) following manufacturer's instruction.

2.5. Free fatty acid (FFA) detection

Serum free fatty acid levels were measured by converting them into their CoA derivates, which are subsequently oxidized with the concomitant generation of color/fluorescence. C-8 (octanoate) and longer fatty acids were quantified by colorimetric (wavelength 570 nm) method with detection limit of down to 2 μM free fatty acid in the samples (Free Fatty Acid Quantification Kit, BioVision, USA).

2.6. Total RNA extraction and reverse transcription

RNA was extracted from *A. cahirinus* testes using RNeasy Mini Kit (Qiagen, Germany) for RNA purification, while RNA from WAT was extracted using EZ-RNA Kit (Biological Industries, Israel, Beit Haemek Ltd.). Total RNA was quantified by a photometrical method at a wavelength of 260 nm. Quality of RNA was verified by loading 1 μL of total RNA onto a RNA 6000 Nano Chip using the Nano Assay 600 Bioanalyzer (Agilent, Waldbronn, Germany) following the manufacturer's instructions. Single stranded cDNA was generated out of 0.5 μg total RNA using a High Capacity cDNA RT Kit (Agentek, UK). The obtained cDNA was stored (−20 °C) until further analysis.

2.6.1. Receptors detection and primer design

Tissues (testes and WAT) were tested for the expression of aldosterone (nr3c2), leptin (Ob-Rt) and vasopressin (AVP) mRNA receptor genes.

Genbank sequences were downloaded from Evidence viewer (NCBI website).

All primer sets on exon–exon junction site, were designed to have a Tm of approximately 60 °C, a GC content of approximately 50%, and