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A comparative study of reproductive and metabolic responses to administration of exogenous melatonin and aldosterone in xeric and mesic spiny mice populations

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ABSTRACT

A comparative study of reproduction revealed differences between desert-adapted *Acomys russatus* and Mediterranean *Acomys cahirinus* populations with respect to the environmental cues used for reproductive activity. Long day (LD) conditions were noted as initial reproductive cue for both populations. This research is a follow-up affects comparative endocrine and metabolic study in regards to reproduction where LD-acclimated mice were treated with, exogenous aldosterone (ALDO) and melatonin (MLT). Only the reproductive system of *A. russatus* females was significantly affected by both hormones. In *A. cahirinus* females, MLT decreased leptin levels, while in *A. russatus*, a treatment with both hormones increased leptin levels. In *A. russatus* males, MLT affects both reproductive and metabolic functions. However, in *A. cahirinus* males, ALDO and MLT treatments caused an increase in leptin levels, and a decrease in free fatty acid (FFA) levels, respectively. Correlations between leptin and FFA in general were affected by both MLT and ALDO treatments in *A. russatus* males and *A. cahirinus* females. Our results support the general idea, that although the reproductive system of *A. russatus* responded to an osmotic stress, in our case expressed by ALDO treatment, which can be considered as an ultimate signal, where, photoperiod changes are an initial signal.

1. Introduction

Reproduction in mammals is an energetically demanding function and thus, should coincide with the time of the year when the habitat is most rewarding (Vaughan et al., 2000). Responsiveness of physiological variables to changing photoperiods from summer to winter and *vise versa* is an important trait in most temperate-zone mammals. Therefore, animals existing in temperate or boreal environments display extensive seasonal variability in many physiological and behavioral characteristics, in order to assist them to cope with the environmental challenges for instance, in the case of reproduction.

Species occurring in environments where conditions for breeding are unpredictable, such as deserts, are probably less reliant on photoperiod as a proximate cue for reproduction (Bronson, 1985). In order to ensure survival and species continuity in a desert ecosystem it is crucial to adjust reproduction and delivery, to resource availability in the habitat and therefore, other cues may be more useful and reliable for timing reproductive activity. Rainfall is one such factor that is important in desert environments (Noy-Meir, 1973).

A common feature in desert rodents could be the inhibition of reproduction under gametogenesis arrest (Bronson, 1989). The timing of sexual activity provides obvious energy savings with respect to gestation and lactation. Therefore, integration of various environmental cues should determine which species will be adapted to breed at the appropriate time and in such a way so as to survive in xeric environments (Schwimmer and Haim, 2009).

It is accepted by reproductive physiologists that seasonal breeders, whether under long or short-days (LD or SD, respectively) are dependent on the annual fluctuations in photoperiod, expressed by the levels and secretion duration of the neuro-hormone melatonin (MLT), produced and secreted by the pineal gland (Barrett et al., 2003). Pineal MLT is produced at night under dark conditions and is suppressed by light (Geoffriau et al., 1998). In rodents plasma MLT exhibits a daily rhythm, with high levels at night and low levels at day time (Butler et al., 2010). Available evidence indicates that MLT regulates the reproductive function in seasonal breeding mammals through its inhibitory action at various levels of the hypothalamic–pituitary–gonadal (HPG) axis. The pulsatile secretion of gonadotropin-releasing hormone (GnRH), from the GnRH neurons in the hypothalamus, regulates the

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luteinizing hormone (LH) and the follicle-stimulating hormone (FSH) secretion which, in turn, regulates the functional activity of gonads (Glass and Knotts, 1987; Kennaway and Rowe, 1995).

The reproductive axis uses the seasonally dependent MLT rhythms to adjust testicular and ovarian physiology accordingly (Glass and Knotts, 1987). In this scenario the timing of mating is determined by the duration of the gestational period which ensures that the offspring are born when environmental temperatures are favorable and resource availability is high as in spring and early summer, in the Mediterranean ecosystem.

MLT has multiple action sites as evidenced by the MLT receptors which exist among others on reproductive organs; such as ovary, testis and epididymis (Pang et al., 1998). The presence of MLT receptors in these tissues is consistent with the idea that direct MLT acts on different levels of the reproductive system. These multiple level actions of MLT at the hypothalamus, pituitary and gonads form a robust system of photoperiodic control in the timing of an animals' reproduction. Such parameters and feedbacks would guarantee successful gestation and delivery of the offspring at an optimum time of food availability and ultimately favorable conditions for the survival of delivered young. Long-day breeders (*e.g.*, many rodents) are sexually depressed during the winter months, when MLT levels are high with the longest secretion duration.

In unpredictable desert ecosystems, photoperiod is important, only as an initial proximate cue but, for full activation of the system and offspring maximal survival, an ultimate cue is necessary such as the availability of water in the habitat. To date it is well known that rain or water inflow into the habitat can be an ultimate reliable cue which triggers reproduction in some desert and dry grassland adapted species (Shanas and Haim, 2004; Bronson, 2009). Osmotic stress is an important internal signal for inhibiting reproductive activity in the golden spiny mouse *Acomys russatus* (Shanas and Haim, 2004; Wube et al., 2008a) and in a desert adapted population of common spiny mice *Acomys cahirinus* (Bukovetzky et al., 2012a). Furthermore, vasopressin (VP) treatment leads to a significant decrease in a females' body and uterine mass and a significant increase in the number of atretic follicles in the ovaries of *A. russatus* (Shanas and Haim, 2004) and reduction of spermatogenesis in males of this species (Wube et al., 2008b). VP also had a negative reproductive effect on desert-adapted *A. cahirinus* (Bukovetzky et al., 2012b). Therefore, in addition to its osmoregulatory function, VP affects the reproductive system and may be a potential candidate for transmitting the message of water shortage to the reproductive system of desert-adapted rodents which switches off reproduction activity (Shanas and Haim, 2004). Aldosterone (ALDO) is also an osmoregulatory hormone that plays an important role in sodium regulation through the proximal tube of the nephron (Williams and Williams, 2003). ALDO secretion results in extremely potent vasoconstriction, affecting also the genital tract, thus it may also serve as a candidate for controlling reproduction under water deprivation. Angiotensin II receptors were discovered in human (males and females) reproductive tract, suggesting a multiplicity of roles that are unrelated to ALD and angiotensin II primary osmoregulatory functions (Hassan et al., 2006).

Although, water is an important environmental ultimate cue for reproduction of desert adapted rodents, energy resources and food availability in the environment also seem to be a critical signal for reproduction, bearing in mind that food is also a water source for such rodents, which have no access to free water (Jensen, 2006). Therefore, food quality can act as a double signal, for water and energy supply. A hormone candidate for sufficient energy resources can be leptin (Bukovetzky et al., 2012a,c) while serum free fatty acids (FFAs) can indicate the levels of white adipose tissue (WAT) catabolism (Bukovetzky and Haim, 2014).

The possible MLT direct effects on the gonads and involvement of MLT as proximate cue for the breeding season; the effect of ALDO is regulating osmoregulation and levels of plasma leptin, and the effect of FFA as indicators of energy supply leads us to suggest the following hypothesis: *If MLT and ALD have a key role in expressing environmental cues to coordinate reproduction in mammals from xeric habitats, then for desert adapted rodents leptin and FFA serum levels will respond to MLT and ALD treatment by modulating reproduction through the sex hormones.*

The objective of the present study was to compare the metabolic (leptin and FFA) and reproductive (steroid hormones and estrous cycle number) responses of *Acomys* populations from two different ecosystems, xeric and mesic, to MLT and ALD by assessing their affects on metabolism by measuring serum leptin and FFA levels and reproductive aspects by measuring progesterone (PROG) and testosterone (TESTO) serum levels.

2. Materials and methods

The experimental mice were obtained from a colony of desert adapted *A. russatus*, and a colony of mesic (Mediterranean) *A. cahirinus*, kept at the Oranim Campus, University of Haifa. Mice in the colony are descendants of individuals originally captured at the Dead Sea shores (*A. russatus*) and Mt. Carmel (*A. cahirinus*). All acclimated mice were adults aged four months. Mice were maintained at an ambient temperature of 28 ± 2 °C for *A. russatus* and 26 ± 2 °C for *A. cahirinus* (Haim and Borut, 1981; Weissenberg and Shkolnik, 1994) respectively, under a photoperiod regime of 12L:12D. In order to avoid bias results related to body mass (W_b), both control and treated groups had a similar average W_b at the beginning of the experiments. Ethical clearance for the use of animals was provided by The Ethical Committee, University of Haifa. Experiments were carried out between March and November 2010, Ethics form number 159.09.

2.1. Photoperiod acclimation

Twenty six (14 females and 12 males) desert-adapted *A. russatus* with a mean body mass of 43 ± 3 g and twenty eight individuals (14 females and 14 males) mesic *A. cahirinus* with a mean body mass of 41 ± 6 g were acclimated to long day (LD) conditions (16L:8D) inside a climatic cabinet (158 × 77 × 74 cm) (Meditest 600/1300, Austria) for three weeks. Lights were on between 07:00 and 23:00 h, a cool fluorescent light with a dominant wavelength of 470 nm and at an intensity of 450 lx, during the photophase and only a red dim light (697 nm) of 25 lx during the scotophase. Animals were kept in separate plastic cages (35 × 25 × 15 cm). Food (rat pellets, Koffolk, Israel) and 2% agar gel (20 g of dry agar dissolved in 1000 mL of deionized water) as a source of water were provided *ad libitum*. W_b was recorded to the nearest 0.01 g using a digital balance (Sartorius, 5500 Germany).

2.1.1. MLT and ALD treatments

After three weeks of acclimation the tested animals were injected with either an exogenous MLT solution (50 mg/kg) or ALD solution (10 μg/kg) (Sigma, Israel) for 21 days. The control group was injected only with saline solution (0.9% NaCl) in the same concentration also for 21 days.

2.1.2. Estrous cycles

Vaginal smears were taken twice a week. Pasteur pipettes with blunt tips were inserted 2 cm into the vaginal tract and vaginal fluid was extracted. The extract was mounted on a microscopic slide, stained with methylene blue and then observed under a microscope (×100). Estrous was evidenced by the presence of superficial (cornified) cells (Bekyurek et al., 2002; Wube et al., 2008b; Bukovetzky et al., 2012a).

2.1.3. Sampling mice

After 21 days of treatment with either MLT or ALD, the mice were anesthetized with a cocktail of ketamine (10 mg/kg) and Rampoone (100 mg/kg), and sacrificed by decapitation between 10-12 h, 3h after light were on, for all groups, 3 h after lights were on. Serum samples were collected for analyzing sex hormones, leptin and FFA. Gonads were removed and weighed using an analytical scale (1907 MP8