

Diet salinity and vasopressin as reproduction modulators in the desert-dwelling golden spiny mouse (*Acomys russatus*)

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Received 4 October 2003; received in revised form 15 January 2004; accepted 2 March 2004

Abstract

The time for reproduction in mammals largely depends on the availability of water and food in their habitat. Therefore, in regions where rains are limited to definite seasons of the year, mammals presumably will restrict their breeding correspondingly. But while mammals living in predictable ecosystems would benefit by timing their season to an ultimate predictable cue, such as photoperiod, in unpredictable ecosystems (e.g., deserts) they will need to use a more proximate signal. We suggest a mechanism by which water shortage (low water content in plants) could act as a proximate cue for ending the reproductive season. The golden spiny mouse (*Acomys russatus*), a diurnal rodent living in extreme deserts, may face an increased dietary salt content as the summer progresses and the vegetation becomes dry. Under laboratory conditions, increased diet salinity lead to reproductive hiatus in females, notable in imperforated vagina, and a significant decrease in the ovaries, uteri, and body masses. In females treated with vasopressin (VP), a hormone expressed during water stress, the uteri and body masses have decreased significantly, and the ovaries exhibited an increased number of atretic follicles. VP has also led to a significant decrease in relative medullary thickness (RMT) of the kidney. It is thus suggested that VP could act as a modulator linking the reproductive system with water economy in desert rodents, possibly through its act on the energetic pathways.

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Keywords: Water economy; Rodent; Arid; Kidney; Salinity; Relative medullary thickness; ADH

1. Introduction

Reproduction in mammals is a highly energy consuming activity, and thus requires that the time of reproduction coincide with the time of year that the habitat is most rewarding [44]. For many rodents of the temperate latitudes, this may occur during the spring and early summer when food availability is high and ambient temperatures are comfortable. However, in species living in arid or xeric ecosystems summer can be an unfavorable season for reproduction. In these ecosystems, water is a limiting resource, especially for mammals, a group that requires additional quantities of this resource during the lactation period [3]. Small mammals will have an extra burden in this habitat due to their small body size and, therefore, relatively large surface area. While in the winter small mammals may have energetic constraints on reproduction due to loss of heat (e.g., the house mouse [4]), in the summer, the main

limit will be loss of water due to increased action of the cooling processes [11].

One of the physiological responses to water restriction is the release of vasopressin (VP) [30]. VP is an antidiuretic hormone that is produced in the cell bodies of the magnocellular neurons of the supraoptic and paraventricular nuclei in the hypothalamus. It is released to the blood stream from the pituitary gland and travels to the kidney where it triggers the contraction of the arterioles in the glomeruli and reabsorption of water from the collecting ducts. This action of VP in the kidney brings about a decrease in plasma filtration and prevents loss of water from the blood to the urine. VP is thought to have no direct reproductive functions [1], but recent studies hint that it may affect the reproductive system in addition to its osmoregulatory actions. In the monogamous prairie vole (*Microtus ochrogaster*) VP was found to be important for pair bonding [6,48], and VP hypothalamic gene expression increased in both males and females postpartum [45]. The reproductive role of VP in the central nervous system is reflected in the dimorphic VP nuclei [35] and the dimorphic excretion of VP in the brain [9,10,33]. Thus, it is possible that VP secretion is influenced

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by sex hormones. Indeed, in humans, plasma VP is reported lowest during and just after menstruation, when estradiol and progesterone concentrations are low, and high at the time of ovulation when these hormones peak [14]. In rats, both VP secretion and responsiveness to VP were affected by gender and by the estrus cycle of the female [10].

In earlier experiments designed to examine the effect of increased osmotic load on physiological parameters of common spiny mice (*Acomys cahirinus*) [40], we noticed that females that consumed a diet high in salt content had imperforated vaginas (unpublished observations). We therefore hypothesized that the reproductive system could be directly affected by factors that regulate the water balance and osmotic systems.

To study the direct effect of water stress and VP on reproduction we chose to work with the golden spiny mouse (*Acomys russatus*), a small desert rodent that is highly adapted to xeric conditions [19]. Golden spiny mice are omnivorous, feeding on plants and invertebrates, with a special preference for snails as an additional source of water [26]. They breed throughout the year in laboratory conditions; however, in nature they were observed to breed between April and July [26]. Their ability to concentrate urine and their low resting metabolic rates are part of an adaptation to cope with diurnal activity and extreme conditions in their habitat [16,41]. This species has also showed enhanced performance in replenishing VP in the pituitary gland under dry condition [7]. *A. russatus* lives in habitats where free water is not available. Consequently it will depend on preformed water from animals (such as land snails and arthropods) and from the vegetation it consumes. During the summer the vegetation in the desert ecosystem is expected to have increased particle (or osmotic) concentra-

tion due to high evaporation of water and limited water recharge [15,17,25]. This may be especially emphasized in drought years that could sometimes occur successively. Therefore, omnivorous animals such as *A. russatus* that feed on desert plants face an increasing osmotic load as the dry season progresses. Under such conditions, reproduction hiatus may be favorable. In this study we simulated end of summer osmotic loads by challenging the animals with saline solutions [23,37], or by a VP treatment, and followed the response of the females' reproductive tract.

2. Materials and methods

2.1. Animals

Adult female golden spiny mice (*A. russatus*) were obtained from the breeding colony of the Department of Biology, University of Haifa at Oranim and were housed individually in plastic cages (35 × 25 × 15 cm) with sawdust as bedding. Animals were kept at room temperature with 14L/10D light cycle, and were offered rat chow and fresh carrots (which supplied the water needs) when they were not undergoing experimentation. Animals were kept for 40 days before experimentation commenced to establish that they were not pregnant. Only females with perforated vagina were selected for the experiments.

2.2. Effect of increased diet salinity on vaginal opening

Females (n=7) were kept on a diet of rat chow and 2% agar gel (20 g of dry agar dissolved in 1000 ml of deionized water) as a source of water. We simulated osmotic stress that

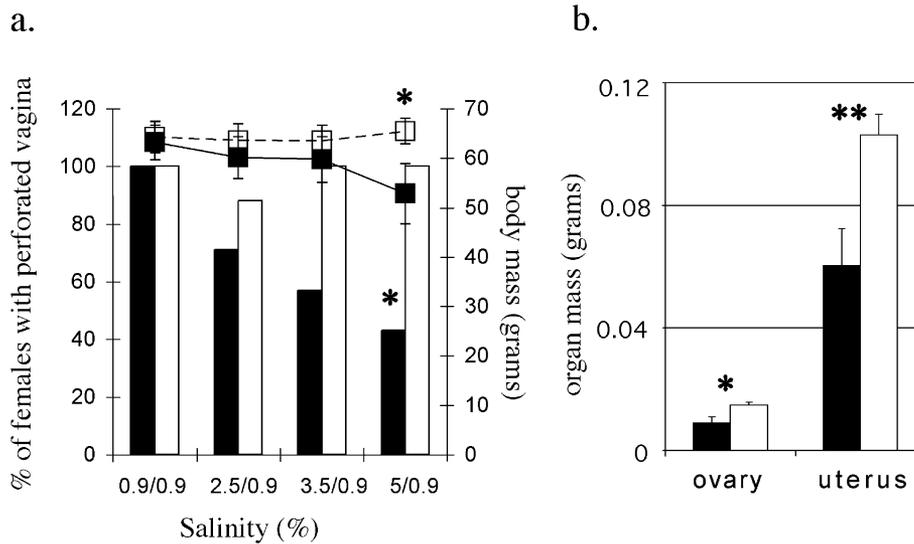


Fig. 1. (a) Percentage of experimental group (■) and control (0.9%) group (□) with perforated vagina at the end of the acclimation period to different dietary salinities. Lines represent the body mass (± S.E.) of females from the experimental (—■—) and control (—□—) groups. (b) Masses of ovaries and uteri (± S.E.) at the end of the experiment in experimental (■) and control (□) groups.

desert animals may experience in the wild by dissolving increasing concentrations of NaCl in the agar. The salt concentration was increased from 0.9% to 2.5%, 3.5%, and 5%. These values have been used in previous studies to increase urine osmolality [37]. Each acclimation period to a specific salinity lasted 10 days, after which the female's vagina was scored as perforated or imperforated. Animals' body mass was examined every 2 days to monitor and prevent sharp decreases in mass. Control females ($n=8$) were kept under the same conditions except that the animals received carrots as a source of water instead of the agar gel. They were scored at the same time as the experimental group. At the end of the last acclimation period five animals from each group were randomly chosen and were euthanized by a short exposure to CO₂. The ovaries and uteri were excised, cleaned of excess adipose and connective tissues, and weighed.

2.3. Effect of VP on reproductive status

We set three groups of females (experimental, recovery, and control, $n=5$ in each) and provided them with rat chow (Koffolk, Israel) and carrot. The animals were injected intraperitoneally every 3 days at 10:00 h with 50 μg VP/kg body mass (Sigma) (experimental and recovery), or with a similar volume of saline (0.05 ml, control) for 3 weeks. The injected VP dose was similar to injected doses used in behavioral studies in mice (e.g. Ref. [8]). Animals of the experimental and control groups were weighed and sacrificed by CO₂ after the 3-week period at about 1000 h. The recovery animals were sacrificed after an additional 3 weeks during which no injections were applied. The ovaries, uteri, adrenals, and kidneys were excised from all animals, cleaned, and weighed. The ovaries were immersed for fixation in

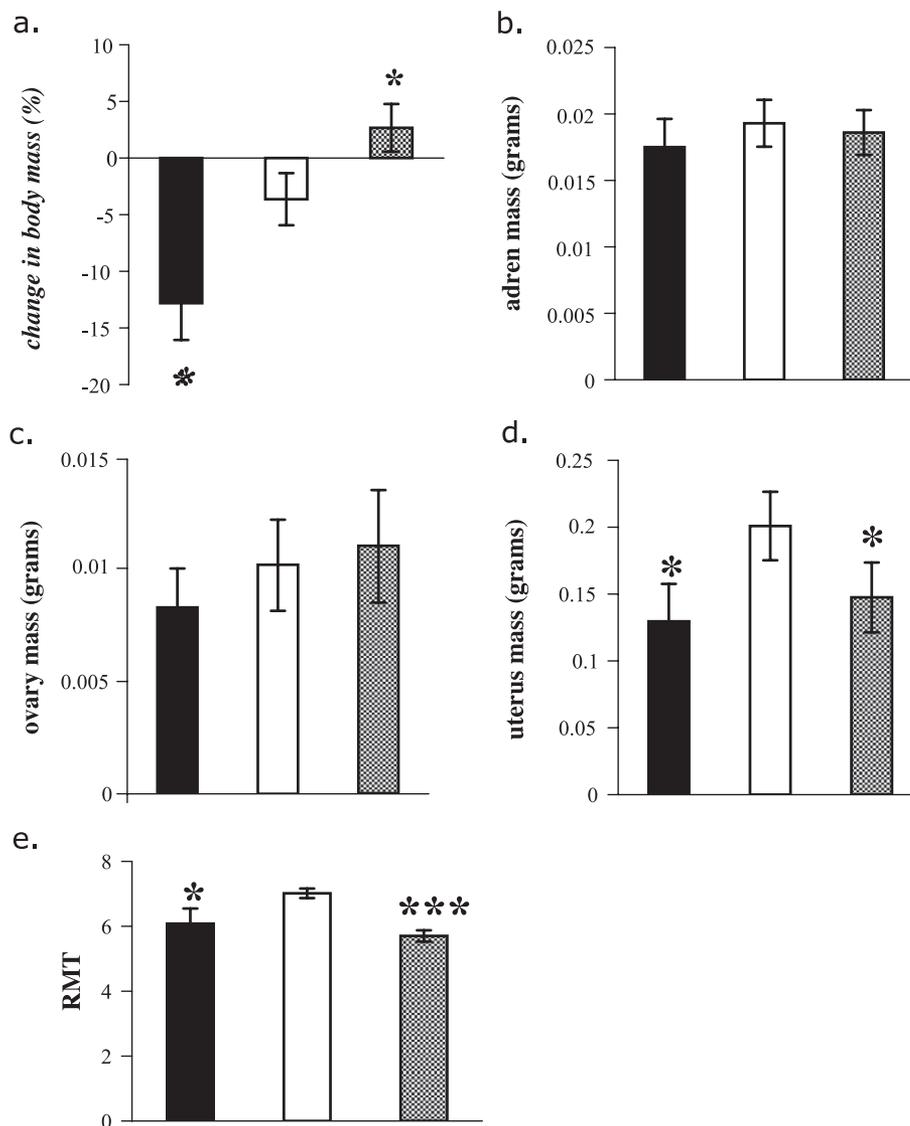


Fig. 2. Change in body mass (a), the mass of the adrenals (b), ovaries (c) and uteri (d) (proportional to body mass), and RMT (e) in experimental (■), control (□), and recovery (▨) groups (* $P<.05$, *** $P<.001$, one-tailed t test).

neutral buffered formalin (NBF) [34] until they were processed for histological examination. The experiments were conducted during the winter and spring of 2001.

2.4. Relative medullary thickness (RMT)

Immediately after the two kidneys were removed and cleaned we measured their length (l), width (w), and thickness (t). They were then cut longitudinally and the width of the medulla was measured with a digital caliper. RMT was calculated for each kidney as: $10 \times (\text{medulla width}) / \sqrt[3]{lwt}$ according to Ref. [43], and for each animal we averaged the RMT of the two kidneys.

2.5. Histological procedures and analysis

The ovaries were removed from the NBF solution after 48 h and placed in phosphate buffer. Subsequently, the tissues were processed and embedded in paraffin, cut serially (8 μm), and stained with hematoxylin and eosin. Every fifth section was mounted and examined under an Olympus CH2 microscope. Follicles of the ovaries were scored and counted as primary, secondary, antral, and corpora lutea. Atretic follicles were defined histologically based on the parameters determined by Byskov [5].

2.6. Statistical analysis

Statistical analysis was carried out using a computer software for Macintosh, Statview 5. Proportions of females with perforated vagina at increasing salinities were compared using Harber proportion correction for χ^2 test [49]. Body masses, organ masses, number of atretic follicles, and kidney RMTs were compared between treated and untreated females using a t test. A value of $P < .05$ was considered significant.

3. Results

3.1. Effect of diet salinity on reproductive status

Increasing the concentration of salt in the water source resulted in a significant ($P < .05$, one-tailed χ^2 test at 5% salinity) decrease of percentage of females whose vagina remained perforated, while no change in vagina perforation was noted in control females (Fig. 1a). Treated animals generally lost body mass, and one of the seven females had to be removed from the experiment after it lost 30% of its initial body mass when acclimated to 3.5% salinity. The treated females had a significant decrease in body mass compared to control ($P < .05$, one-tailed t test, Fig. 1a). The mass of the ovaries and of the uteri decreased significantly in the experimental group ($P < .05$ and $P < .01$, respectively, one-tailed t test; Fig. 1b). Moreover, ovaries and uteri weighed less in treated animals compared to control when their mass was calculated as a percentage of body mass

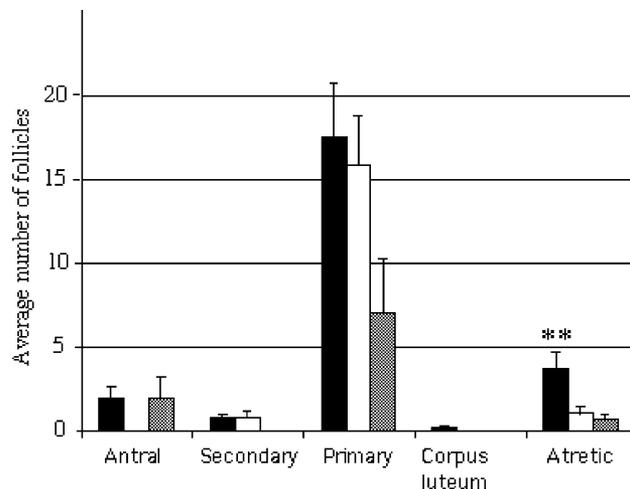


Fig. 3. The number of follicles in the ovaries of experimental (■), control (□) and recovery (▨) groups (** $P < .01$, one-tailed t test).

(ovaries, experimental vs. control: 0.018% vs. 0.024%; uteri, experimental vs. control: 0.12% vs. 0.17%).

3.2. Effect of VP on reproductive status

VP treatment did not affect the vaginal perforation of the treated females, as only one of the females in the experimental group had an imperforated vagina at the end the experiment. It appears that VP treatment leads to a significant decrease of females' body mass ($P < .05$, one-tailed t test, Fig. 2a), but adrenal glands and ovaries had no change in their mass (Fig. 2b and c). A significant decrease in the mass of the uteri was noted in the experimental group (Fig. 2d).

Body mass of the recovery group regained and even exceeded its initial values. The recovery group did not differ from the experimental group with respect to adrenal and ovary mass. Uterus mass of the recovery group was not regained and, similar to the experimental group, was significantly lower than the control.

In the ovaries of VP-treated females, there were significantly more atretic follicles as compared with control or with the recovery group (Fig. 3, $P < .01$, one-tailed t test). However, no difference was found in the number of viable follicles among the groups.

3.3. Effect of VP on kidney morphology

RMT decreased significantly ($P < .05$, one-tailed t test) in females treated with VP (Fig. 2e) and continued to decrease in the recovery group.

4. Discussion

The golden spiny mouse is well adapted to xeric conditions [16,41]. When dietary salt content increases,

the mouse will respond through a significant increase of urine osmolality and a decrease in resting metabolic rate and body mass [37]. Our study shows that although *A. russatus* is well adapted to survive high salt concentrations in its diet, its fecundity will be impaired, as reflected by the increased number of females with imperforated vagina.

In desert habitats, when the summer progresses, the ambient conditions for reproduction become unfavorable for mammals. It is thus advantageous for desert species to halt their reproductive activity at this time, and thereby save those limited resources, such as water, for life-supporting activities. For example, the sexual activity of female fat sand rats (*Psammomys obesus*) was found to be correlated with rainfall and rain days [13]. Water can be a limiting resource for rodents living in mesic ecosystems as well. The California mouse (*Peromyscus californicus*) that is abundant in chaparrals and woodlands (mesic environments with long, dry periods) reproduces during the rainy winter season [47]. In this species, photoperiod and food availability failed, while water availability succeeded in regulating its unusual timing of reproduction [31]. In several mesic species, a mid-summer breeding hiatus has been observed. In deer mice (*Peromyscus maniculatus*), water restriction significantly reduced testicular mass and spermatogenic activities when compared to mice with free access to water [27]. Water restriction was also effective in reducing reproductive activity in male prairie vole (*M. ochrogaster*) and in male wild mice (*Mus musculus*) but not in the laboratory strain [28]. Therefore, it has been suggested that water availability can serve as a reliable cue as well as a proximate factor for reproduction hiatus [27–29,42]. However, the mechanism for this response has never been revealed.

The severe ambient conditions during the summer forces rodents like *A. russatus* to consume increasing amounts of vegetative products or to prey on land snails and other invertebrates to satiate their water requirements. However, increased vegetation consumption during the summer may lead to an increase in salt consumption, leading to increased blood osmolality. When we simulated end of summer conditions in the laboratory by providing high salt content in the animals' diet, we observed that reproduction was impaired, but in addition we also observed a decrease in body mass (see also Refs. [19,37] for *Acomys*, and Refs. [19,32,39] for other species). It is therefore possible that the observed alterations in reproductive parameters found in this study were not caused directly by the increased blood osmolality but resulted from decreased body mass, which has previously been correlated with fecundity [3,20]. Alternatively, as increased blood osmolality stimulates peripheral and central release of VP [18,21,33], it is possible that VP itself will convey the message of increased blood osmolality to the reproductive system.

Administering VP to *A. russatus* females for 3 weeks resulted in reduced body mass, reduced uteri mass, and a significant increase in the number of atretic follicles in the ovaries. These results suggest that VP could serve as a good candidate for sending the message of water shortage to the reproductive system. Several studies reported the occurrence of VP receptors in the reproductive tract [22,24]. However, oxytocin receptors are also present in the reproductive tract [24]; thus the structural similarity of these two hormones could possibly cause the injected VP to act upon these receptors as well.

Reproductive failure is a classic symptom of stress [2], and VP could also act on the reproductive system as a classic stressor. This hormone can stimulate adrenocorticotrophic hormone (ACTH) secretion [2] that stimulates synthesis and release of glucocorticoids. It was noted, for example, that in stressed cows VP showed the same blood concentration time course as do other known stressors, such as corticotropin releasing hormone (CRH) [12]. We did not find differences in the mass of the adrenal gland between VP-injected animals and control, suggesting that VP did not dramatically affect the hypothalamic–pituitary–adrenal axis.

Surprisingly, VP caused a significant decrease in body mass. Therefore, it is likely that VP can affect the reproductive system through the energetic pathways. Future experiments will have to examine whether the decrease in body mass is through reduced food consumption or perhaps increased metabolic rate. Robinson et al. [36] reported similar effects on reproduction of a related hormone, oxytocin. However, no record was given to the effect of oxytocin on body mass in that study, and therefore the mode of action of oxytocin on the reproductive system also needs to be elucidated.

This experiment also revealed a significant reduction in the RMT of the kidney as a result of VP injections. Apparently, the time for recover was not sufficient for the kidney medulla to regain its initial size, as in the recovery group the RMT of the kidney continued to decrease. The size of the kidney's medulla can serve as a reliable sign of the kidney performance. Thick medullas are typical of desert animals [11,38,46] having elevated capabilities to concentrate urine by building an osmotic gradient along an extended loop of Henle. The addition of exogenous VP increases water absorption in the collecting duct. This increase in water absorption can compensate for a shrink in the length of the loop of Henle.

In conclusion, we describe here two novel effects of VP and suggest that water can serve as a proximate cue to the reproduction season through the action of VP.

Acknowledgements

We thank Ms. Arian Wallach for her assistance in maintaining the animal colony.

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