Simulated Drought Influences Reproduction in Male Prairie Voles

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NELSON, R. J., D. FRANK, S. A. BENNETT AND C. S. CARTER. Simulated drought influences reproduction in male prairie voles. PHYSIOL BEHAV 46(5) 849-852, 1989.—The environmental factors that arrest breeding in prairie voles during the middle of the breeding season are unknown. The role of water availability on reproductive function was examined by limiting water intake to 50% of ad lib water consumption for 10 weeks. At autopsy, testicular, epididymal and seminal vesicle masses were reduced in water restricted males as compared to animals with ad lib access to water. Body mass was also reduced in water restricted males. Plasma testosterone levels and the number of testicular and epididymal sperm were significantly reduced in water restricted voles as compared to animals drinking water ad lib, but plasma levels of luteinizing hormone were unaffected. Taken together, these data suggest that reduced water availability can inhibit male prairie vole breeding.

Voiles Water restriction Seasonal reproduction Seasonality Male reproduction Testis function

PRAIRIE voles (Microtus ochrogaster) occupy grassland habitats throughout much of the midwestern United States (2). These herbivorous rodents are exposed to typical continental variation in climate. Prairie voles breed on a seasonal basis with most offspring produced during spring and summer and relatively few pregnant females observed during the late autumn and winter [reviewed in (10)]. Breeding during the summer is not continuous; two summer peaks of breeding, separated by a mid-season hiatus, have been reported for prairie voles (6, 8, 16). Laboratory studies of rodent seasonal breeding have addressed the proximate factors leading to the cessation of reproductive activities in autumn and vernal reproductive recrudescence. However, few experiments have addressed the proximate factors that regularly stop breeding during the summer breeding season (1, 10, 12, 17, 18).

There are several intrinsic and extrinsic factors that could mediate the mid-summer break in reproductive activities in prairie voles. The breeding hiatus could reflect exhaustion among individual females after two or three consecutive litters. It is also possible that the break in reproductive activities may simply represent the time for the young of the current season to mature after the demise of the over-wintering animals. Alternatively, the cessation in breeding could be directly mediated by environmental factors. Likely extrinsic factors include food quality or quantity, ambient temperature or water availability. These factors could influence reproduction in males, females or both sexes. Photoperiod is unlikely to be important in affecting the timing of the midseason breeding hiatus; prairie voles breed at high rates when maintained in laboratory photoperiods of 16 hr light/day (9).

Previous work on other rodent species suggests that reduced water intake significantly curtails reproductive function (1, 11-13, 18). Several studies of natural populations of prairie voles and other rodents suggest that lack of water contributes to the cessation of breeding during the summer (1). Laboratory studies also indicate a direct effect of water restriction on reproduction independently from other cues (13). We examined in the laboratory the role of water availability on the reproductive physiology of male prairie voles. Water intake was decreased by half for ten weeks. Reproductive function was impaired by water inanition, as indicated by reductions in organ weights, spermatogenic activity, as well as plasma levels of testosterone. Body mass also decreased after water restriction. These data support the contention that reduced summer water availability in the field may be sufficient to arrest reproduction in prairie voles.

METHOD

Seventy adult (>75 days of age) male prairie voles (Microtus ochrogaster ochrogaster) were obtained from our laboratory colony. Animals were housed with same sexed siblings from weaning until the onset of the study; thereafter, males were housed individually in polypropylene cages (28 × 17 × 12 cm) and provided with food (Agway Prolab RMH 1000 supplemented with Quaker rolled oats) and tap water ad lib. Ambient temperature was

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maintained at 23±2°C and animals were exposed to a programmed daily LD 14:10 (14 hr light/day; lights on 06.00 hr EST) photoperiod.

Daily water intake was measured for 15 consecutive days for all voles. Twenty males continued to receive unlimited water supplies. Fifty voles were provided with 50% of their average individual ad lib water intake. Body mass was measured and males were examined for pigmented, scrotal testes at the onset of the study. At 10 weeks, all animals were anesthetized with methoxyflurane (Metofane; Pitman-Moore), weighed and a blood sample was obtained from the orbit of the eye (14). Voles were immediately injected with a lethal dose of sodium pentobarbital. The masses of paired testes, epididymides, as well as seminal vesicles were recorded at autopsy.

Spermatogenic activity was assessed by counting the number of sperm-shaped cells (including elongated spermatids) in paired testes and epididymides. For this purpose, organs were dissected and separately transferred to a blender. Testes and epididymides were homogenized for 30 and 45 seconds, respectively, in 25 and 50 ml 0.15 M sodium chloride plus 0.05% Triton-X 100 (Sigma Chemical Co., St. Louis, MO) and 0.25 M thimerosal (Sigma). A sample of the resulting homogenate was removed and placed on a hemocytometer grid under phase-contrast microscopy. Duplicate counts were made for each sample. This technique has been more fully described elsewhere (4).

Blood samples were centrifuged, plasma was separated from the cells and stored at −80°C until assayed for luteinizing hormone (LH) and testosterone. Plasma testosterone titers were determined in 50 μl samples using a double antibody radioimmunoassay procedure previously validated for small volumes of blood plasma (5). First antibody to testosterone was purchased from Radioassay Systems Laboratories (Rao-R181) (Carson, CA). All blood plasma samples were assayed at the same time. The within assay coefficient of variation was 8.2% for eight samples of plasma obtained from a pool of breeder male prairie voles. Plasma concentrations of immunoreactive LH were assayed in duplicate 50 μl samples following validated procedures for small rodents (4). Standard curves, including 10 levels of rLH-RP-2 ranging from 0.1–50 ng/tube, were run in triplicate. Final values were expressed in terms of ng equivalents of the reference preparation (rLH-RP-2) per ml of vole blood plasma. All plasma samples were measured in the same assay.

Data were analyzed by Student's t-test for independent samples. Differences between group means were considered statistically significant when p<0.05.

RESULTS

Chronic restriction in water consumption impaired reproductive function in male prairie voles. Seminal vesicles, as well as paired testes and epididymal masses, were reduced in water restricted males as compared to animals with ad lib access to water (p<0.001, p<0.05 and p<0.01, respectively) (Fig. 1). Both epididymal and testicular sperm numbers were significantly depressed in the water restricted mice as compared to animals drinking water ad lib (p<0.01 and p<0.05, respectively) (Fig. 2). Plasma levels of testosterone were depressed by more than 60% by water restriction (p<0.001). Blood plasma LH levels were unaffected after ten weeks of water restriction (p>0.05).

Body mass was reduced among water restricted males (p<0.01) (32.67±3.93 g vs. 42.67±3.79 g). Daily average ad lib water intake was 8.6 cc/day. Daily average water intake of restricted animals was 5.3 cc/day.

DISCUSSION

Sustained water inanition impaired reproductive function among male prairie voles. Seminal vesicle as well as paired testicular and epididymal masses were significantly depressed by restricted water intake. The sperm contents of the testes and epididymides were lower among water restricted voles as compared to animals with ad lib access to water. Plasma testosterone levels also followed this pattern; voles with restricted water intake had lower plasma testosterone titers than voles with unlimited access to water.
Body mass was reduced among water restricted males. Decreased gonadal size could be due to reduced food intake in response to water inanition. Food intake was not measured in the present study; however, previous data for *Peromyscus maniculatus* and *Mus musculus* indicate that relative daily food intake does not differ between restricted and nonrestricted males (11,13).

Although the reduction in sperm numbers was substantial, 20,000,000 epididymal sperm may be sufficient to impregnate female prairie voles. Studies on rats and hamsters suggest that similar reductions in epididymal sperm numbers interfere with fertility (7,15). Regardless of the number of sperm available for ejaculation in the epididymides, reproductive behavior may be curtailed during drought.

Presumably, low testosterone levels would not support reproductive behavior. In a separate study (Nelson and Bennett, unpublished data), voles were subjected to 50% water restriction for ten weeks, paired with females for 6–9 days, videotaped and the number of litters recorded. No water restricted males mated; however, only 30% of the males receiving ad lib water sired offspring. We are not confident from these results that reduced plasma testosterone levels observed after water restriction in these voles translates into decreased mating behavior. Further studies are necessary to sort out the influence of reduced water intake on male and female reproductive behavior and physiology.

Relative to the poor conditions associated with winter, droughts are often short-lived. Rodents that inhibit their reproductive systems in response to short day lengths require 30–45 days to resume spermatogenesis. It is probably not adaptive to completely regress the reproductive system during water shortages because of the temporal constraints of testicular regression and subsequent recrudescence. Male rodents may suppress androgen production and avoid the energetic costs associated with androgen-dependent behaviors. Spermatogenesis may be maintained at reduced levels during water shortages, rather than stopped, to limit the time required to begin breeding when conditions improve.

The present data suggest that reduced water availability may contribute to the cessation of reproductive activities during the summer observed among natural populations of voles.

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