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The Existence of a Puberty Accelerating Pheromone in the Urine of the Male Prairie Deermouse (Peromyscus maniculatus bairdii)

LINDA G. TEAGUE[1] and ERIC L. BRADLEY[2]

Laboratory of Endocrinology and Population Ecology,
Department of Biology,
College of William and Mary,
Williamburg, Virginia 23185

ABSTRACT

These experiments were designed to test for the presence of a puberty accelerating pheromone in the prairie deermouse *(Peromyscus maniculatus bairdii).* Body weight, total reproductive weight, ovary and oviduct weight and uterus weight were measured in animals exposed to water, fresh urine of intact males, frozen urine from intact males and urine from castrate males. Corpora lutea and maturing follicles were evaluated in animals exposed to water and urine from intact males.

Exposure to fresh or frozen urine from intact males for 14 days resulted in significant increases in mean ovarian and uterine weights relative to animals exposed to water. Urine from castrate males did not produce any increase in reproductive tract weight relative to water. Significantly more corpora lutea were present in animals exposed to urine from intact males than in those exposed to water. It was concluded that a pheromone which accelerates puberty in the young female deermouse is present in the urine of the male deermouse and is not destroyed by freezing.

INTRODUCTION

Recent studies have indicated the *Mus musculus* male exerts considerable influence on the rate of sexual maturation of the female. Exposure of groups of 6–8 females at 21, 28 or 35 days of age to an adult male results in an acceleration of vaginal opening and first estrus in proportion to the length of exposure (Vandenbergh, 1967). Oral-nasal application of male urine also produces an acceleration of vaginal opening and estrus (Colby and Vandenbergh, 1974) and uterine hypertrophy (Bronson and Maruniak, 1975; Vandenbergh et al., 1975).

While the presence of pregnancy block and estrous synchrony pheromones have been established in the prairie deermouse *(Peromyscus maniculatus bairdii),* the presence of a puberty accelerating pheromone has not been confirmed. The purpose of this investigation was to determine if such a pheromone is present in the deermouse.

METHODS AND MATERIALS

Animals used in this study were drawn from a colony of prairie deermice which had been maintained in the laboratory for approximately 13 years without sib matings. Animals from the field have been added annually when possible to preserve genetic heterogeneity. All animals received food and water *ad libitum.* Temperatures in the animal rooms ranged from 18–28°C. Air was exchanged 5–10 times/h.

Pairs mated for litter production were drawn directly from the colony at a minimum of 90 days of age. They were maintained on a regime of 12 h of light (4, 40 watt fluorescent bulbs on at 0730 h) and 12 h of darkness and housed in opaque plastic cages (18 × 28 × 11 cm) on wood shavings. One week prior to expected parturition, the females were isolated and moved to a room containing no adult males. Their light regime was changed to 14 h of light (lights on at 0700) and 10 h of dark. The day after birth, all litters were sexed and reduced to 4 or 5 including at least 1 male. At 21 days of age, the young were weaned, females were weighed, isolated and randomly assigned to treatment groups. No attempt was made to restrict the weight range of the females used.

Urine Collection

Animals used for collection of intact male urine pools were a minimum of 120 days of age and of proven fertility. They were maintained on 12 h of light (4, 40 watt fluorescent bulbs on at 0730 h) and 12 h of darkness. Males were housed opposite females in one side of metal no-contact cages (13 × 24.4 × 24.4 cm) with grid floors. The hardware cloth partition permitted mutual reception of visual, auditory and olfactory cues. All urine collections were made

[1] Present Address: Medical College of Virginia, Richmond, Virginia

[2] Address for reprints.

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daily from 0800 h–1200 h. All food was removed from the cage and a piece of fine mesh copper screen was mounted below the grid floor during collection to prevent fecal contamination of the urine. Each cage was mounted 2 cm above a plexiglass plate. Urine from the 8 males was picked up directly from these plates with a disposable glass pipet, pooled and used immediately or frozen for future use.

Five males which were a minimum of 90-days-old were castrated 45 days prior to their use for collection of urine. All castrates were housed in a single undivided metal cage (13 × 24.4 × 24.4 cm). Other maintenance and collection procedures were identical to those described above.

Treatment Procedure and Tissue Collection

Each female was exposed daily to 0.05 ml of water or urine according to the predetermined schedules described below. Each test substance was applied directly to the oro-nasal groove with a 1 ml syringe. In most cases, the applied material was at least partially ingested so that gustatory as well as olfactory stimulation was possible.

At the end of the treatment period, each animal was weighed to the nearest 0.1 gm. Subsequently, the animal was rapidly anesthetized and then killed with diethyl ether. All mice were placed in 10% formalin and after at least 72 h, the uterus, ovaries and oviducts, were removed as a single unit and cleaned, blotted and weighed. The ovaries and oviducts were then removed and weighed as paired organs. Uterine weights were determined by subtraction. All organ weights were measured to the nearest 0.01 mg on an electrobalance (Cahn) interfaced with a programmable calculator (Wang 700B).

Design

The experimental treatments were as follows:

Experiment 1: Two groups of females received applications of water or of fresh urine from intact males for 6 days. Treatments began at 22 days of age and ended at 27 days of age. Animals were sacrificed at 28 days of age.

Experiment 2: Two groups of females received applications of water or of fresh urine from intact males for 14 days. Treatments started at 21 days of age and ended at 34 days of age. Animals were sacrificed at 35 days of age.

Experiment 3: Four groups of females received applications of water, fresh urine from intact males, frozen urine from intact males or fresh urine from castrate males for 13 days. Treatments began at 21 days of age and ended at 33 days of age. Animals were sacrificed at 34 days of age.

Histology

Six randomly selected ovaries from groups treated with water or fresh urine from intact males of Experiment 3 were embedded in Paraplast, serially sectioned (10 μm) and stained with hematoxylin and eosin. The total number of follicles of types 5a, 5b, 6, 7 and 8 (Pederson and Peters, 1968) and the number of corpora lutea were determined for these ovaries. Atretic follicles were not counted. The ovarian elements were counted at 100X magnification.

Statistics

Comparisons among body weights, organ weights and ovarian elements were made using a Kruskal-Wallis test. If overall significance was found, urine treatment groups were compared with the water treatment group using a Mann-Whitney U test to investigate the relationships between all pairs of treatments in experiments showing overall significance. A probability of less than 0.05 was considered significant in all cases.

RESULTS

No significant differences in mean body weights or mean organ weights between groups treated with water or urine from intact males were observed in Experiment 1 after 6 days of treatment (Table 1). In experiment 2, where animals were treated for 14 days, body weights at weaning and sacrifice were not significantly different, but mean total reproductive unit weight, mean ovary and oviduct weight and mean uterus weight of the group treated with urine from intact males significantly exceeded those of the group treated with water ($P<0.005$, 0.025 and 0.005, respectively; Table 1).

Comparisons between the various treatments in Experiment 3 show that no significant differences in mean body weights at weaning were observed in any treatment groups (Table 1). However, at sacrifice, the mean body weights of the group treated with fresh urine from intact males and the group treated with frozen urine from intact males significantly exceeded the mean body weight at sacrifice of the group treated with water ($P<0.05$ in each case) and no significant difference in mean body weight at sacrifice existed between treatments with water or with male urine of castrates (Table 1). Also, the mean total reproductive unit weight, mean ovary and oviduct weight and mean uterus weight of the treatments with fresh urine of intact males, or frozen urine significantly exceeded those of the group treated with water ($P<0.025$ or less, 0.025 or less and 0.005, respectively). The mean total reproductive unit weights, mean ovary and oviduct weights of the group treated with urine of castrates did not differ significantly from those of the group treated with water (Table 1), but were significantly less than the values for groups treated with fresh or frozen urine ($P<0.025$ or less).

In addition, the mean body weight at sacrifice did not differ between the groups treated with fresh male urine or frozen male urine, but these both were significantly heavier

TABLE 1. Mean ± SEM of body weights at weaning and sacrifice, total reproductive unit weights, ovary and oviduct weights and uterus weights for animals in Experiments 1, 2 and 3.

Treatment	No. of animals	Weaning weight (g)	Sacrifice weight (g)	Total reproductive unit weight (mg)	Ovary and oviduct weight (mg)	Uterus weight (mg)
			Experiment 1 — treated for 6 days (D22–D27)			
Water	8	9.1 0.3	11.7 0.5	12.13 1.09	6.40 0.65	5.72 0.59
Intact male urine	9	9.3 0.3	12.3 0.5	16.94 3.94	7.87 1.23	10.15 3.66
Kruskal-Wallis		NS	NS	NS	NS	NS
			Experiment 2 — treated for 14 days (D21–D34)			
Water	13	9.3 0.4	13.1 0.5	19.48 4.22	7.18 0.90	12.30 3.48
Intact male urine	13	9.7 0.4	13.5 0.4	36.20 4.99	10.56 1.02	25.63 4.52
Kruskal-Wallis		NS	NS	P<0.005	P<0.025	P<0.005
			Experiment 3 — treated for 13 days (D21–D33)			
Water	9	8.8 0.5	10.8 0.3	14.79 2.35	6.79 0.82	8.00 1.56
Intact male urine	10	9.2 0.3	11.8 0.3*	25.41 3.18****	9.40 0.84**	18.25 3.60****
Frozen male urine	10	9.1 0.2	11.8 0.3*	32.35 5.29**	9.94 0.94***	22.71 6.29****
Castrate male urine	10	9.2 0.1	10.9 0.2	14.93 2.60	6.11 0.39	8.81 2.44
Kruskal-Wallis		NS	P<0.05	P<0.05	P<0.01	P<0.05

*Significantly different from the water treatment at P<0.05, **0.025, ***0.01, and ****0.005.

($P<0.05$) than the groups treated with urine of castrates. Mean reproductive organ weight for all parameters measured did not differ significantly between the groups exposed to fresh male urine or frozen male urine (Table 1).

Analysis of the histology of the ovarian elements in 6 randomly selected animals from both the water or the groups treated with intact male urine (Experiment 3) revealed no significant differences in the number of follicles of type 5a, 5b, 6, 7 or 8. However, a significantly greater ($P<0.05$) number of corpora lutea was found in animals exposed to intact male urine (1.0 ± 1.0 vs 6.7 ± 2.1). Of these animals, 67% of the ovaries exhibited corpora lutea while only 17% of the ovaries of animals exposed to water possessed corpora lutea.

DISCUSSION

The results of this study have confirmed the presence of a factor in the fresh or frozen urine of intact male deermice which promotes sexual development in female deermice as measured by uterine and ovarian weight relative to females treated with water. This factor was shown to be testis dependent because it was not present in the urine from castrated males. These observations are consistent with those of Vandenbergh et al. (1975) who demonstrated a testis dependent factor that was stable in frozen urine from *Mus musculus*.

In addition, the histological examination of the ovaries of the deermice in this study clearly indicated that by 35 days of age, few animals (17%) had spontaneously produced corpora lutea, whereas animals treated with urine had an increased number of corpora lutea per animal and more animals possessed corpora lutea. Although luteinization of unovulated follicles cannot be ruled out, it is most likely that these corpora lutea represented increased frequency of ovulation in the groups treated with urine.

Although the pheromone activity we have described here involves the acceleration of sexual maturation in young females, it is quite possible that this activity is promoted by the same male urinary substance that was reported earlier by Bronson and Marsden (1964) and shown to synchronize the estrous cycle of adult females of this same species. Of course, further clarification of this point must await the physical characterization of the urinary pheromone(s).

The deermice used in this study were maintained as an outbred strain with sib matings not permitted and wild mice were added to the colony when possible. This no doubt was responsible for the great variability observed in the weaning weights and final organ weights. Possibly, it was because of this variability that we could develop no correlations between the weaning or final body weights and the organ weights attained for each animal in response to urine treatment. The presence of the urinary factor accelerating sexual maturity in this genetically heterogeneous population supports the suggestion that such a factor is present in the urine from wild male deermice.

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