Z. Säugetierkunde 58 (1993) 31–37

ISSN 0044-3468

Influence of the hormonal state of female Mongolian gerbils (*Meriones unguiculatus*) on urinary chemosignals stimulating scent-marking behaviour of males

By B. Probst and Heidrun Schönheiter

Zoologisches Institut/Tierphysiologie der Universität Tübingen, Tübingen, FRG; und Lehrstuhl Tierphysiologie der Universität Bayreuth, Bayreuth, FRG

Receipt of Ms. 24. 2. 1992
Acceptance of Ms. 6. 4. 1992

Abstract

Investigated the influence of urine from female Mongolian gerbils (*Meriones unguiculatus*) at different endocrine conditions on urinary chemosignals that stimulate male scent-marking behaviour. The scent-marking behaviour of male Mongolian gerbils is increased by presence of females. This increase is mediated by non-volatile polypeptides excreted in urine by mature females. In the present study the scent-marking behaviour stimulating efficacy of female urine originating from ovariectomized and hormone-treated females and also from mature females during different estrous states was determined. Urine from ovariectomized females and ovariectomized females treated only with progesterone did not increase the scent-marking frequencies of males in the open-field tests. After treatment of ovariectomized females with estradiol or estradiol together with progesterone female urine significantly stimulated the scent-marking frequencies of males. The natural estrous cycle of Mongolian gerbils appeared rather unpredictable in individual females, while 4–6 day intervals were dominating in the complete sample. Only urine from proestrous females stimulated scent-marking behaviour of males while urine from other estrous states was ineffective.

Introduction

Fertile male Mongolian gerbils (*Meriones unguiculatus*) display an androgen-dependent scent-marking behaviour (Thiessen 1968), during which they deposit the secretions from a specialized ventral scent gland onto prominent objects in their environment. In fertile males the frequency of this scent-marking behaviour is highly dependent on the availability of chemical signals excreted by females (Probst 1985a). Based upon that increase in scent-marking frequency we developed a bioassay for the chemical signal: Males kept without females scent-mark on a low basal level that is approximately doubled when female urine or an appropriate test sample is applied to the nares of the males (Probst and Lorenz 1987).

Mature female gerbils undergo a spontaneous estrous cycle of 4 to 7 days (Marston and Chang 1965; Barfield and Beeman 1968; Vick and Banks 1969). The present experiments therefore investigated the influence of the sexual status of the donor females on the scent-marking stimulating efficacy of their urine. Tested were urine samples from ovariectomized females with and without hormone treatment, and from intact females at different states of their estrous cycle.

Material and methods

Animals and housing

Mongolian gerbils (*Meriones unguiculatus*) were obtained from our breeding colony. Males were housed in individual macrolon cages with wire mesh tops (cage size: 37 × 20 × 15 cm). Females were

kept in groups of two or three per cage. All gerbils were maintained on a 12:12 hours light-dark cycle (lights on from 02.00 to 14.00 hours) with constant room temperature (22 ± 1 °C) and relative humidity (55 %). Gerbil food pellets (Altromin, Lage, Germany) and tap water were available ad lib.

Behavioural observation

The experimental males were kept in a separate room where only males were present. Their scent-marking behaviour was observed daily in the same room in an open-field apparatus (Thiessen 1968), modified as described earlier (60 × 60 cm, transparent walls 27 cm, 9 marking pegs; Probst 1985b). For scent-marking tests, individual males were placed inside this open-field and all occurrences of scent-marking behaviour during 5 minutes were counted. All observations were made during the third hour of the dark phase of the light-dark cycle. A dimmed fluorescent bulb provided sufficient illumination for observation of the animals after a short period of adaptation.

Bioassay procedure

To determine the efficacy of different urine samples to increase scent-marking activity, each male received 20 µl of the respective urine sample onto its nares twice per day. The samples were given at least 7 hours before the behavioural tests and immediately following the tests. Evaluated were 3 trials per male starting after 5 days of urine treatment. The stimulating effect of female urine on male scent-marking behaviour is completely vanished after 2 weeks without treatment. Between different treatments a phase of 7 to 18 days with no treatment was placed, during that the decline of scent-marking activity back to the basal level was monitored. This experimental design allowed us to compare marking activities from the same males under different experimental conditions thus avoiding the comparison between different treatment groups. At the start and after completion of an experiment all males were tested with pooled female urine to verify that no habituation of the response had occurred during the series.

Statistical analysis

The basal and pooled urine stimulated marking activities determined at the start and after the respective experiment were compared using Wilcoxon matched-pairs signed-ranks test. Since there were no significant differences (Tab. 1), all basal marking activities during the tests were averaged to one individual's basal marking activity. In addition, the response to pooled urine is not significantly different at the start compared to the end of the experiment (Table). For further statistical analyses, the average scent-marking activity during the respective treatment period was computed for every male. The effects of different treatments in both experiments were then evaluated by parametric analysis of variance (ANOVA), because Bartlett (Chi^2 [df = 4] = 6.65 and 2.02) and Hartley (F-max [df = 4] = 2.29 and 1.87) tests indicated homogeneity of variances ($p = 0.15$ and 0.75). When applicable, significant treatment effects were evaluated using the Tukey honest significant difference (HSD) test. Interactions between consecutive tests were prevented by the randomized treatment sequence and the intermediate unstimulated period, during that the decline of marking activities to the basal level was monitored. The significance level was set to $p < 0.05$.

Experiment 1: Influence of urine from ovariectomized and hormone-treated females

Nine adult females were ovariectomized under Ketanest (Parke, Davies and Co., Berlin, Germany) anesthesia. Starting six weeks after surgery urine was collected from these females (OVX) by placing

Comparison of basal and pooled urine stimulated marking activities (mean ± SEM) obtained at the start (no. 1) and the end (no. 2) of the respective experiment

NS indicates not significantly different marking activities using Wilcoxon matched-pairs signed-ranks test ($p > 0.05$)

	Experiment 1 (N = 15)		Experiment 2 (N = 15)	
Basal no. 1	8.52 ± 1.06	NS	15.28 ± 2.01	NS
Basal no. 2	8.53 ± 1.07		14.87 ± 2.29	
Pool no. 1	14.98 ± 1.45	NS	27.24 ± 3.05	NS
Pool no. 2	15.47 ± 1.45		27.82 ± 2.95	

every female individually inside a beaker for some minutes. Following this sampling period, the females were randomly assigned to one of three groups. One group (E2) received estradiol benzoate for 10 days (5µg once per day). Another group (P) received progesterone for ten days (0.5 mg once per day). Urine was collected on days 5 to 10 of hormone treatment in E2 and P groups. The last group (E2 + P) was treated with estradiol (5 µg; 48 and 24 before sampling) and progesterone (0.5 mg; 3 hours before sampling); this treatment reliably elicited receptive behaviour.

Individual urine samples were kept frozen at −40 °C until the end of the sampling period, when urine from equal treatment groups was pooled. Fifteen experimental males received these pooled urine samples in randomly assigned sequence.

Experiment 2: Influence of urine from different natural estrous states

Twentysix females, 6–7 months of age, were used as urine donors. For daily urine collection, every female was individually placed inside a beaker for a maximum of 2 minutes. The urine sample was collected and immediately stored frozen at −40 °C. Subsequently the estrous state of this female was determined by placing her inside the cage of a sexually active male. A female was rated in behavioural estrus when the male caused the display of lordosis behaviour (Davies et al. 1974; Burley 1980). Immediately after the estrous state was determined, the female was removed from the male to prevent pregnancies during the experiments.

After completion of the sampling period (103 days) the urine samples from all females were classified according to the following estrous states:

DE = diestrus: no receptive behaviour at least one day before and one day after this sampling day
PE = proestrus: the day before behavioural estrus was observed
E = estrus: the female was receptive and displayed lordotic behaviour
ME = metestrus: the day following behavioural estrus

The pooled urine samples of the above classes were dialysed against demineralized water for 1 hour, which reduced osmolarity from more than 3000 mosmol/l to 200 mosmol/l (Knaur Micro-Osmometer, Knauer, Germany) to prevent any possible impairment of the nasal mucosa during the experiments.
Fifteen males were testes in randomized sequence with urine from the different estrous states.

Results

Experiment 1: The scent-marking activities of the 15 test males remained unaffected during treatment with urine from ovariectomized female conspecifics (Fig. 1). Also, urine from ovariectomized females treated with progesterone failed to stimulate male scent marking

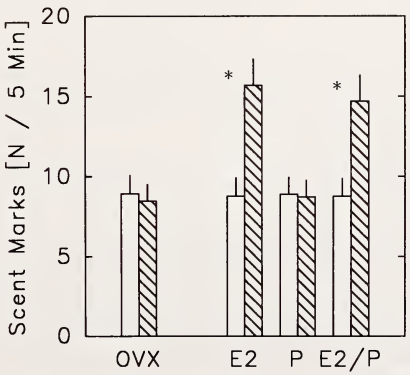

Fig. 1. Influence of urine from ovariecomized females (OVX) and females treated with progesterone (P), estradiol (E2) or progesterone and estradiol (E2/P) on male marking activity. Indicated is the basal marking activity (open columns) and the marking activity after at least 5 days of urine treatment (striped columns) (mean ± SEM, n = 15, *: $p < 0.01$)

behaviour. Urine from ovariectomized females treated daily with estradiol or with a combination of estradiol (HSD: $p < 0.01$) and progesterone (HSD: $p < 0.02$) significantly increased the scent marking levels of the males (ANOVA: $F [4; 70] = 7.4$; $p < 0.001$).

Experiment 2: A total of 109 cycles were observed in the 26 females (2–8 cycles per female). Cycles with 4 to 6 days of length clearly dominated with more than 75 % of all cycles observed. However, 7 females displayed anestrous periods from 7 to 23 days of length. Urine from such anestrous periods was not used for stimulation of the males.

Analysis of variance for the scent-marking activities revealed a statistically significant difference in scent-marking response to urine from different estrous states (ANOVA: $F [4;70] = 4.07$, $p < 0.001$). Post-hoc comparisons showed that urine from proestrous females significantly increased male scent-marking activities (HSD: $p < 0.05$; Fig. 2). There was no significant influence of estrous, metestrous and diestrous urine on male scent-marking frequency.

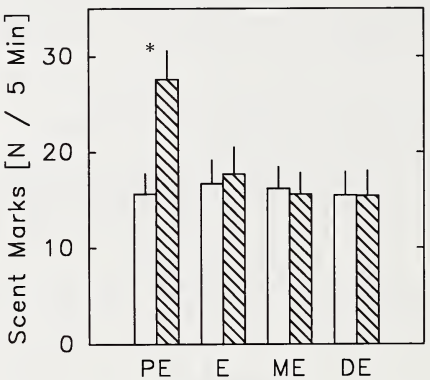

Fig. 2. Influence of urine from intact females in different estrous states on male marking activity. Indicated is the basal marking activity (open columns) and the marking activity after at least 5 days of urine treatment (striped columns) (mean ± SEM, n = 15, *: $p < 0.01$). PE = proestrus, E = estrus, ME = metestrus, DI = diestrus

Discussion

The experiments reported here investigated the influence of the sexual state of females on the efficacy of their urine to increase the scent-marking activities of male conspecifics. During the first experiment the sexual status of the females was manipulated using ovariectomized and hormone-treated females. For the second experiment urine was obtained from mature females having a natural estrous cycle. Effects of the endocrine and sexual condition of the female urine donors on the males' scent-marking activities were found in both experiments.

In a number of experiments before starting these series, we evaluated different methods for determination of female receptivity. The typical sequence of different cell types found in vaginal smears during the estrous cycle in other rodents [rat *Rattus norvegicus*]: Long and Evans 1922; hamster (*Mesocricetus auratus*): Kupperman 1944; Scharmann et al. 1988; mouse (*Mus musculus*): Bronson et al. 1966) was not observed in gerbils (see also Marston and Chang 1965). The change of cell types in vaginal smears, therefore, cannot predict the day of estrus in gerbils. Also, the urinary excretion of free estradiol did not show the cyclic pattern (Fenske and Probst, data not shown) that can be found in other female mammals during the ovarian cycle (Bonney and Setchell 1980; Khatkatay et al.

1988; Härter and Erkert 1991). The hormonal regulation of the chemical signal by estradiol as found in experiment 2 is not detectable as a rhythmic pattern of the excreted amount of estradiol in cycling females. However, the estradiol metabolites in the urine were not determined in the present study. Therefore, the regulatory function of estradiol in cycling females can not be rejected by this method. In the present study the intervals between consecutive heats were unpredictable in individual females. This is in contrast to Burley et al. (1980), who observed rather constant 4 day cycles in individual females. Therefore, daily pairing with a sexually active male, which proved to be the most reliable determination of the estrous state, was performed throughout the experiments. These sexually active males usually responded to female receptive behaviour in less than 60 seconds with sexual behaviour.

Sexual and endocrine conditions exert effects on behaviour in various species. Male golden hamsters (*Mesocricetus auratus*) flank marked at low rates in soiled cages of females on estrous and one day postestrous, and at high rates when females were on non-estrous days (Johnston 1977). The effect of the endocrine condition of the female on male behaviour in direct encounters was also investigated by Agren and Meyerson (1977). In their study, male Mongolian gerbils (*Meriones unguiculatus*) displayed most marking when females were ovariectomized and not treated with hormones, while estradiol-treated females as well as estradiol- and progesterone-treated females elicited only 50 % of the marking scores as compared to the untreated females. In our experiments, female urine stimulated male scent-marking activity when females were one day before estrous or when ovariectomized females were treated with estradiol or a combination of estradiol and progesterone. The effect on scent-marking, however, is not comparable between these studies. Agren and Meyerson (1977) as well as Johnston (1977) observed an immediate change in the behaviours displayed during an encounter or under changing experimental conditions. During the treatment of male gerbils with female urine their scent-marking activities do not increase immediately (Probst 1985a). After at least three days of continuous treatment the scent-marking activities achieve a higher level. This higher level is maintained after withdrawal of the stimulus for one or two days. It then takes several days until the marking levels steadily decrease back to the basal marking activities. This means, the internal mechanism that sets the marking activity of a male to a specific level is modulated by the chemical signals from urine of proestrous females. Using the terminology for the classification of pheromones (Vandenbergh 1983) for these experiments (Agren and Meyerson 1977; Johnston 1977), the immediate changes in specific behaviours would be caused by "signalling pheromones". In contrast to that, the long-lasting increase in marking activity induced by urinary chemosignals in male gerbils would be caused by "primer pheromones". In contrast to the effects of other primer pheromones, female urine causes a reversible increase of scent-marking behaviour in male gerbils, and not an irreversible developmental or morphological change (Vandenbergh et al. 1975; Drickamer 1986).

Although the physiological control of scent-marking behaviour is well known in gerbils (Thiessen and Yahr 1977) the biological functions of this behaviour are still unclear (Baran and Glickman 1970; Fullenkamp et al. 1985). The increased marking frequency could provide olfactory familiarity of a particular male that functions to mitigate a female's aggressiveness during encounters (Daly 1977). In a recent field study from Inner Mongolia, Agren et al. (1989) found 6–8 times more marking behaviour during sexual interactions than before. As demonstrated by the present results, the efficacy of female urine to increase scent-marking in males depends on the sexual state of the female. This all indicates an important function of male scent-maring behaviour in the sexual context.

Acknowledgements

This work was supported by a grant of Deutsche Forschungsgemeinschaft (Pr 285/1-1) to B. P. We thank Sünje Meyer and Daniela Hirzel for help during the experiments.

Zusammenfassung

Einfluß des hormonellen Status weiblicher Mongolischer Rennmäuse (Meriones unguiculatus) auf die Steigerung des Markierverhaltens von Männchen durch chemische Signale im Urin

Untersucht wurde, welchen Einfluß des Sexualstatus von weiblichen Mongolischen Rennmäusen (*Meriones unguiculatus*) auf im Urin enthaltene chemische Signale hat, die das Markierverhalten von Männchen steigern. Die Häufigkeit des Markierverhaltens von männlichen Mongolischen Rennmäusen wird durch die Gegenwart von Weibchen gesteigert. Dieser Anstieg wird durch schwerflüchtige Polypeptide bewirkt, die mit dem Urin fertiler Weibchen ausgeschieden werden. Für diese Untersuchungen wurde Urin von ovarektomierten und hormonbehandelten Weibchen sowie Urin aus bestimmten Ovarialzyklusphasen von fertilen Weibchen gesammelt. In einem Biotest wurde anschließend bestimmt, inwieweit diese Urinproben die Markieraktivität von Männchen steigern können. Urin ovarektomierter Weibchen und von ovarektomierten Weibchen bei Behandlung mit Progesteron führte dabei zu keiner Steigerung der Markieraktivität. Wurden ovarektomierte Weibchen mit Östradiol oder Östradiol und Progesteron behandelt, so führte ihr Urin zu einer signifikanten Zunahme der Markieraktivität bei Männchen. Die Zykluslänge war bei einzelnen Weibchen sehr variabel, obwohl insgesamt 4–6 Tage lange Zyklen vorherrschten. Nur der Urin proöstrischer Weibchen führte zu einer signifikanten Steigerung der Markieraktivität, während Urin der anderen Östrusstadien unwirksam war.

References

Agren, G.; Meyerson, B. J. (1977): Influence of gonadal hormones and social housing conditions on agonistic, copulatory, and related socio-sexual behaviour in the Mongolian gerbil (*Meriones unguiculatus*). Behav. Process. **2,** 265–282.

Agren, G.; Zhou, Q.; Zhong, W. (1989): Ecology and social behaviour of Mongolian gerbils, *Meriones unguiculatus*, at Xilinhot, Inner Mongolia, China. Anim. Behav. **37,** 11–27.

Baran, D.; Glickman, S. E. (1970): "Territorial marking" in the Mongolian gerbil: A study of sensory control and function. J. Comp. Physiol. Psychol. **71,** 237–245.

Barfield, M. A.; Beeman, E. A. (1968): The oestrous cycle in the Mongolian gerbils, *Meriones unguiculatus*. J. Reprod. Fertil. **17,** 247–251.

Bonney, C. R.; Setchell, K. D. R. (1980): The excretion of gonadal steroid during the reproductive cycle of the owl monkey (*Aotus trivirgatus*) J. Steroid. Biochem. **12,** 417–421.

Bronson, F. H.; Dagg, C. D.; Snell, G. D. (1966): Reproduction. In: The biology of the Laboratory Mouse. Ed. by L. L. Green. New York: McGraw-Hill.

Burley, R. A. (1980): Pre-copulatory and copulatory behaviour in relation to stages of the oestrous cycle in the female Mongolian gerbil. Behaviour **72,** 211–241.

Daly, M. (1977): Some experimental tests of the functional significance of scent-marking by gerbils (*Meriones unguiculatus*). J. Comp. Physiol. Psychol. **91,** 1082–1094.

Davies, H. N. Jr.; Estep, D. Q.; Dewsbury, D. A. (1974): Copulatory behavior of Mongolian gerbils (*Meriones unguiculatus*). Anim. Learn. Behav. **2,** 69–73.

Fullenkamp, A.; Fisher, R. B.; Vance, R. A.; Duffey, K. A. (1985): The failure to demonstrate avoidance of ventral gland odors in male Gerbils (*Meriones unguiculatus*). Physiol. Behav. **35,** 763–765.

Härter, L.; Erkert, H. G. (1991): The length of the ovarian cycle does not depend on the circadian period length on common marmosets, *Callithrix j. jacchus*. In: Primatology today. Ed. by A. Ehara, T. Kimura, O. Takenaka, and M. Iwamoto. Amsterdam: Elsevier Science Publishers. Pp. 431–434.

Johnston, R. E. (1977): Scent marking by male Golden hamsters (*Mesocricetus auratus*). Z. Tierpsychol. **37,** 75–98.

Khatkhatay, M. I.; Sankolli, G. M.; Meherji, P, K.; Chowdhury, V.; Joshi, U. M. (1988): Excretion of estrone-3-glucuronide in urine in spontaneous and induced ovulatory cycles. Int. J. Fertil. **333,** 181–187.

Kupperman, H. S. (1944): Sexual cycle and dimorphic pigmentation in the golden hamster (*Cricetus auratus,* Waterhouse). Endocrinology **35,** 225.

Long, J. A.; Evans, H. M. (1922): The oestrous cycle in the rat and its associated phenomena. Mem. Univ. Calif. **6,** 1–148.

Marston, J. H.; Chang, M. C. (1965):The breeding, management and reproductive physiology of the Mongolian gerbil (*Meriones unguiculatus*). Lab. Anim. Care **15,** 34–48.

Probst, B. (1985a): Female urinary chemosignals influence scent-marking behaviour in male Mongolian gerbils (*Meriones unguiculatus*). Z. Naturforsch. **40c**, 936–938.
— (1985b): Individual marking activities not reflected by respective testosterone levels in male gerbils. Physiol. Behav. **34**, 363–367.
Probst, B.; Lorenz, M. (1987): Increased scent marking in male Mongolian gerbils by urinary polypeptides of female conspecifics. J. Chem. Ecol. **13**, 851–861.
Scharmann, W.; Nagel, P.; Heller, A. (1988): Oestrus phases of the Syrian hamster. Z. Versuchstierkde. **31**, 276–280.
Thiessen, D. D. (1968): The roots of territorial marking in the Mongolian gerbil: A problem of species-common topography. Behav. Res. Meth. Instru. **1**, 70–76.
Thiessen, D. D.; Yahr, P. (1977): The gerbil in behavioural investigations. Austin: University of Texas Press.
Vandenbergh, G. H. (1983): Pheromones and Reproduction in Mammals. New York: Academic Press.
Vandenbergh, G. H.; Whitsett, J. M.; Lombardi, J. R. (1975): Partial isolation of a pheromone accelerating puberty in female mice. J. Reprod. Fertil. **43**, 515–523.
Vick, L. H.; Banks, E. M. (1969): The oestrous cycle and related behaviour in the Mongolian gerbil (*Meriones unguiculatus* Milne-Edwards). Comm. Behav. Biol. **3**, 117–124.

Authors' addresses: Dr. Bodo Probst, Zoologisches Institut, Tierphysiologie, Universität Tübingen, Auf der Morgenstelle 28, D-7400 Tübingen, FRG; Dipl.-Biol. Heidrun Schönheiter, Lehrstuhl Tierphysiologie, Universität Bayreuth, D-8580 Bayreuth, FRG