



ARTICLES

High level of circulating testosterone abolishes decline in scent attractiveness in antigen-treated male mice

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We hypothesized that a decrease in the attractiveness of odours from infected male mice, *Mus musculus* could be caused by either parasite-induced changes in host metabolism (parasite-dependent pathway) or activation of the immune defence system (immune-dependent pathway). Activation of the immune defence system by nonreplicated antigens (sheep red blood cells, SRBC) is sufficient for a decrease in both scent attractiveness and the concentration of plasma testosterone in male mice. Since the scent attractiveness of male mice depends on androgen levels, we studied the olfactory effect of SRBC in sham-operated, gonadectomized and testosterone-treated male mice of the outbred strain ICR. SRBC administration resulted in a decline in both sexual odour attractiveness and concentration of urinary proteins in sham-operated control males. The effect of SRBC on concentration of urinary proteins was abolished by stabilizing the testosterone level in gonadectomized and androgen-treated males. At the same time, only the high dose of testosterone was sufficient to maintain the scent attractiveness in SRBC-treated males at the pretreatment level.

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The ability to recognize contagious animals is important for behavioural defence against infectious diseases (Loehle 1995; Kiesecker et al. 1999). Studies of male mice, *Mus musculus*, showed that infection with the enteric protozoan parasite *Eimeria vermiformis*, the nematode *Heligmomoides polygyrus* or influenza virus decreased male odour attractiveness in an olfactory mate choice test (Kavaliers & Colwell 1993, 1995a, b; Kavaliers et al. 1997; Penn et al. 1998). The ability to avoid parasitized mating partners is necessary for both individual protection from sexual transmission of contagious diseases and preventing progeny from inheriting low-resistance genes. For example, female rats, *Rattus norvegicus*, can distinguish the odour of males infected with the tapeworm *Heminolepis diminuta*. Although this parasite is not directly transmissible among rats, the ability to choose uninfected males results in the progeny inheriting resistance to tapeworm (Willis & Poulin 2000).

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Alteration of chemical signals in infected animals could result either from parasite-dependent modification of the composition of volatile components in urine and sweat, or from modification of host metabolism caused by induction of an immune–neuroendocrine response (Penn & Potts 1998; Moshkin et al. 2001, 2002; L. A. Gerlinskaya & M. P. Moshkin, unpublished data). Artificial antigenic challenge is an appropriate approach for elucidating the role of the immune–neuroendocrine response in the alteration of chemical signals found in infected animals. Injection with sheep red blood cells (SRBC) has no toxic effect, but it stimulates an immune–neuroendocrine response in animals similar to that caused by parasitic antigens (Besedovsky & Sorkin 1975; Korneva & Shkinek 1988; Barnard et al. 1998). Therefore, the use of SRBC allows one to estimate the contribution of immune defence to the complex behavioural effects of infection (Kavaliers & Colwell 1993, 1995a, b; Kavaliers et al. 1997; Penn et al. 1998; Willis & Poulin 2000).

SRBC used as an antigenic challenge decreases plasma testosterone, scent attractiveness and reproductive success in male mice (Moshkin et al. 2002). In European blackbirds, *Turdus merula*, treatment with SRBC makes the bill colour used in mate choice paler and decreases the reproductive success of males (Faivre et al. 2003).

In rodents, attractiveness of males correlates positively with secretion of androgens (Ferkin et al. 1994), which affects marking behaviour, production of sexual pheromones, and the concentration and fractional composition of major urinary proteins (Churakov & Novikov 2000; Marchlewska-Koj et al. 2000). Urinary proteins are known to bind and slowly release signalling volatiles such as thiazole and brevicomin (Humphries et al. 1999; Sharrow et al. 2002). Both the concentration and the fractional composition of excreted urinary proteins shape the individuality and attractiveness of the male scent marks (Cavaggioni et al. 1987; Bacchini et al. 1992; Robertson & Beynon 1993; Singh 2001; Beynon & Hurst 2003).

Since androgens are known to affect attractiveness of males and their concentration drops under antigenic challenge, we hypothesized that the observed decline in both scent attractiveness and concentration of urinary proteins induced by activation of the immune system would be abolished upon stabilization of the circulating androgens in castrated or testosterone-treated males. To test this, we studied the effects of antigenic challenge with SRBC on scent attractiveness, and the concentration and fractional composition of urinary proteins in gonadectomized, testosterone-treated and sham-operated male mice.

METHODS

Animals and Procedures

We obtained laboratory mice of the outbred ICR strain, 8–10 weeks old, from stock in the State Research Center of Virology and Biotechnology Vector, Novosibirsk, Russia, in two batches with an interval of 2 months between them. The first batch consisted of 76 males and 35 females and the second of 59 males and 25 females. The mice were kept for 2 weeks in single-sex groups of five or six animals in standard plastic cages (35 × 21 cm and 9 cm high) with sawdust as nesting material at room temperature (20–22°C) under a reversed light:dark cycle (14:10 h, lights off at 0900 hours) and ad libitum food pellets (Zoomir, St Petersburg, Russia). Males and females were kept in separate rooms with separate ventilation. After 2 weeks of adaptation to the new environment (reversed photoperiod) males were caged singly. At 10–12 weeks of age, males from the first batch were divided randomly into four experimental groups with 19 males in each group.

Control males (SO) were sham operated. Three other groups were castrated (GDX) or castrated and injected with low and high doses of testosterone (GDX + 1T and GDX + 10T). With the second group of mice, we repeated the experimental protocol with one exception. Group GDX + 1T was excluded because the dose of testosterone was too low for morphophysiological traits to recover in gonadectomized males (see Table 1 in the Results). These males were divided into three groups: control ($N = 20$), GDX ($N = 20$) and GDX + 10T ($N = 18$).

Gonadectomy was done under ether anaesthesia. Two weeks after gonadectomy, males of the GDX + 1T and GDX + 10T groups were injected subcutaneously with 0.2 ml of Omnadren-250 (Jelfa, Jelenia Gora, Poland) diluted in pitch oil. Omnadren-250 consists of four testosterone ethers (testosterone propionate 0.03 g/ml, testosterone phenylpropionate 0.06 g/ml, testosterone isocaproate 0.06 g/ml, testosterone caprin 0.1 g/ml). These testosterone ethers support a stable increase in plasma testosterone over 4 weeks because of their different rates of diffusion into blood and half-life time. Two doses of Omnadren-250 were used: 1 mg and 10 mg per animal for GDX + 1T and GDX + 10T, respectively. Sham-operated and GDX groups were injected with pitch oil as controls. Five days later all males were injected intraperitoneally with 0.5 ml of SRBC (2×10^8 cells). Faecal samples were collected from the males' cages during the night before SRBC treatment for assessment of testosterone concentration.

Five days after SRBC injections male mice were killed by cranium-cervical dislocation under ether anaesthesia. Lymphoid organs (spleen and thymus) and androgen-dependent organs (preputial glands and seminal vesicles) were weighed. Splenic antibody (plaque)-forming cells (AFC) were measured by local haemolysis of the sheep erythrocytes (Cunningham 1965), following the protocol described in Moshkin et al. (1998). The spleen was removed and carefully disrupted in 5 ml of ice-cold medium 199 (pH 7.3; Biopreparat Co., Novosibirsk, Russia). A suspension of cells was prepared with a 5-ml syringe to filter tissue through a 1-mm mesh screen. The resulting suspension was diluted 1:200 with 5% acetic acid and we counted nucleated cells using a Gorjaev haemocytometer. The reaction mixture consisted of 500 µl of spleen cell suspension, 500 µl of washed SRBC (4×10^9 erythrocytes/ml) and 500 µl of lyophilized guinea pig

Table 1. Mass of endocrine glands and immunocompetence organs in groups of sham-operated (SO), gonadectomized and testosterone-treated male mice

	SO	GDX	GDX + 1T	GDX + 10T
Testosterone in faeces (ng/g)	7.33 ± 0.53 (24) ^b	3.75 ± 0.45 (26) ^c	4.59 ± 0.45 (16) ^{b,c}	27.37 ± 4.66 (29) ^a
Body mass (g)	31.49 ± 0.43 (39)	31.44 ± 0.48 (39)	31.43 ± 0.79 (19)	31.84 ± 0.50 (38)
Preputial glands (mg)	50.43 ± 2.43 (39) ^a	31.35 ± 1.66 (39) ^c	39.58 ± 4.54 (19) ^b	46.25 ± 2.03 (38) ^{a,b}
Seminal vesicles (mg)	112.58 ± 7.21 (39) ^a	18.81 ± 2.84 (39) ^c	47.48 ± 5.25 (19) ^b	102.4 ± 5.00 (38) ^a
Spleen (mg)	131.3 ± 4.8 (39) ^b	191.0 ± 6.9 (39) ^a	183.4 ± 17.9 (19) ^a	146.3 ± 6.0 (38) ^b
Thymus (mg)	59.69 ± 3.15 (39) ^b	95.90 ± 5.17 (39) ^a	61.05 ± 5.40 (19) ^b	42.68 ± 2.76 (38) ^c
Antibody-forming cells	29 140 ± 4354 (39)	34 530 ± 4382 (39)	34 160 ± 8984 (19)	28 850 ± 4692 (38)

Values are means ± SE. Numbers of males are in parentheses. GDX: castrated; GDX + 1T: castrated and treated with a low dose of testosterone; GDX + 10T: castrated and treated with a high dose of testosterone. Different superscript letters indicate means that are significantly different (least significant difference test: $P < 0.05$).

serum (Immunogen Co., Perm, Russia) resolved with 1 ml of isotonic sodium chloride solution. Cunningham chambers were prepared from glass microscope slides, loaded with 200 μ l of reaction mixture, and incubated for 2 h at 37°C before plaques were counted. The numbers of AFCs were calculated per mice.

Olfactory Test

We used sawdust from male cages that had been soiled for 5 days before and 5 days after the males had been injected with SRBC. Samples of soiled bedding were put in glass vials and frozen immediately after collection and kept at -20°C for either 2 or 7 days before odour attraction tests.

Female mice were caged singly and moved to the test room (isolated from other animals) for 4–6 h before an olfactory test. Two hours before a test, we took a vaginal smear to determine the stage of the oestrous cycle. We tested the oestrous and nonoestrous females 2–4 h after light off. Five minutes before the test, we placed the cage with the female into the plastic box (50 \times 40 cm and 40 cm high) with an inflow of fresh air. Two wire-mesh baskets (2 \times 2 \times 2 cm) with soiled bedding (ca. 5 ml) were placed in the opposite corners (above a lid) of a front wall of a cage with the test female for 2 min. Frozen bedding was thawed to room temperature just before the test. One basket contained soiled bedding collected before SRBC treatment and another contained bedding collected from the same male after SRBC treatment. Standard mouse cages (35 \times 21 cm and 9 cm high) were fitted with longitudinal metal screens (8 cm), which divided the front of the cages into two open compartments. We randomly chose the left or right compartment for the odour stimulus. The baskets were about 17 cm apart and were introduced when a female was near the distant wall of a cage. We recorded sniffing when the female's nose approached within 1 cm of a basket. The time spent sniffing was recorded for each scent sample. We calculated differences between the times (DT) that females spent sniffing soiled bedding collected after and before SRBC administration to estimate the antigen-induced changes in scent attractiveness. Samples of soiled bedding collected from intact male mice with a 5-day interval and stored in the freezer for either 2 or 7 days are equally attractive to females (Moshkin et al. 2001).

Urinary Protein Assays

Since odour attractiveness of urine depends on the concentration of urinary proteins (Humphries et al. 1999; Marchlewska-Koj et al. 2000; Beynon & Hurst 2003), we measured protein concentration and protein fractional composition in urine samples collected before and after SRBC administration. We collected urine samples from sham-operated (30 samples) GDX (32 samples) and GDX + 10T (32 samples) groups 1 day before and 5 days after SRBC administration. Urine was collected by gentle abdominal massage while the male was held over a glass tube. No urine samples took longer than 1 min to

collect. Protein concentration was measured in urine just after sampling by the Bradford assay (Bradford 1976). The remaining urine was immediately frozen and kept at -20°C .

Gel filtration chromatography of the urinary proteins was performed on a liquid chromatograph Agilent 1100 with a TSK-GEL G3000SW column equilibrated with 45 ml of 10 mM phosphate buffer (pH 7.2), with a UV detector (260 nm) and ChemStation software (Agilent Technologies GmbH, Waldbronn, Germany). Urine (20 μ l) was applied to the column and eluted from it with the same buffer at a flow rate of 0.6 ml/min. We used bovine albumin (67 kDa), myoglobin from horse heart (18 kDa) and bacitracin (1.45 kDa) as protein molecular weight markers. Chemicals and reagents were obtained from Sigma, St Louis, U.S.A.)

Testosterone Assays

Fresh faeces were collected from male cages during the night (from 0900 to 1900 hours) on the fifth day after the males had been injected with Omnodren-250 or pitch oil. Faecal samples were dried at 30–40°C and stored in a closed tube at room temperature. We measured the concentration of testosterone in dry faeces by radioimmunoassay using Sigma antibodies (Rabbit Anti-Testosterone) and Amersham labelled hormones ([1,2,6,7- ^3H] Testosterone, Amersham, Boston, U.S.A.). The steroids were extracted as follows: dry faeces (60 mg) were homogenized in double distilled water (3 ml) with a glass grinder. After centrifugation, supernatants were collected and stored at -20°C until assayed. Steroid hormones were extracted from 0.4 ml of supernatant with 3 ml of ethyl ether. We transferred 2 ml of the extract into the fresh tube, vacuum dried it at 55°C and resuspended the residue in 100 μ l of 0.01 M phosphate buffer, pH 7.4 (Gerlinskaya et al. 1993; Rogovin et al. 2003).

Following the Sigma protocol, we added 500 μ l of 10-fold diluted antiserum to the tubes with resuspended samples. After vortexing and 30 min incubation at room temperature, 100 μ l of ^3H -testosterone diluted in phosphate buffer was added and samples were incubated again for 1 h at 37°C. After cooling on ice, bound fractions of testosterone were separated from unbound fractions by 10 min incubation with 200 μ l of dextran-coated charcoal suspension and centrifugation at 2000 g for 15 min at 4°C. The supernatants from each tube were added to scintillation cocktails and counted on a Liquid Scintillation Counter (Beckman, Albrville, U.S.A.).

The extraction yield was checked for every set of assays using [^3H]-labelled testosterone and varied from 70 to 76%. The assays were validated for use with mice faecal steroid extracts by determining accuracy and parallelism (Chard 1978). Sensitivity of the assay was determined from the 95% confidence interval of zero standards (5 pg/tube). The inter- and intra-assay variations were 7.9 and 6.2, respectively. To determine the parallelism, we prepared a five-point, two-fold dilution series of faecal samples in phosphate buffer and compared them with the standard curve of testosterone. There were no significant differences between the slope of the standard curve

and the slopes of lines generated from faecal samples of assayed mice.

Statistical Analysis

We used one-way ANOVA to evaluate the effects of gonadectomy and testosterone treatment on the morphological data (mass of lymphoid and androgen-dependent organs). Between-group differences were assessed by the least significant difference test. We assessed concentration of the urinary proteins by two-way ANOVA with experimental groups (control, GDX and GDX + 10T) and injection with SRBC as factors. The influences of the groups and physiological condition of females (oestrous/non-oestrous) on both the total time that the female spent sniffing two alternative scent stimuli and DT were assessed by two-way ANOVA. Paired Student's *t* test was performed for comparison of scent attractiveness, level of urinary proteins and their fractional composition determined before and after injection with SRBC. Data are reported as mean \pm SE.

Ethical Note

The study was done with permission of the Ethical Committee of the Institute of Systematics and Ecology of Animals, Novosibirsk. Since we spent less than 5 min on the gonadectomy, ether was chosen as a fast and controllable anaesthetic. *Before surgery, the mice were put into a transparent hermetic box with ether-impregnated cotton under the wire-mesh floor until they were in deep sleep, assessed visually. Sleeping animals were positioned on the operation table with the head in a beaker connected to an anaesthetic machine adapted for use with mice, which delivered air-ether mixture to the beaker. An assistant reduced the flow rate of the air-ether mixture when respiration rate looked to be slow or arrhythmic. We did not lose any animals during and after gonadectomy. Gonadectomized males from groups GDX + 1T and GDX + 10T were singly injected with Omnadren-250 solute in pitch oil as the recommended solution for the steroid hormones (Mashkovskii 1978). This gentle oil did not induce any irritation or inflammation in the injection site. Since personal identification of soiled bedding was necessary in our experimental design we caged male mice singly for 24 days.

RESULTS

Effects of Gonadectomy and Testosterone

Gonadectomy resulted in a decrease in faecal testosterone and in the mass of the preputial glands and seminal

*Editorial footnote: The Guidelines for the Treatment of Animals in Behavioural Research and Teaching cite evidence that ether is generally not a recommended method of anaesthesia/euthanasia. However, given its historical widespread use, and the lack and cost of suitable alternatives in the country where this research was conducted, its use was considered acceptable on this occasion.

vesicles, and an increase in the mass of immunocompetence organs, such as the spleen and thymus (Table 1). The low dose of Omnadren-250 in GDX + 1T was not sufficient to bring faecal testosterone and mass of the preputial glands, seminal vesicles and spleen up to control levels (Table 1).

Male mice injected with 10 mg Omnadren-250 (GDX + 10T) had higher levels of faecal testosterone than SO males. However, the testosterone concentration in GDX + 10T males did not exceed the natural variation of faecal androgens (2–58 ng/g) observed in ICR mice kept in different social environments (Moshkin et al. 2003), and these males had a similar mass of androgen-dependent and immunocompetence organs, except for the thymus which was significantly lighter than in SO mice. The high degree of variation in concentration of faecal androgens is consistent with the natural variation of androgens measured in blood of male mice and other rodents (Nelson et al. 1975; Heywood 1980).

In all cases humoral immune response to thymus-dependent antigens (SRBC) estimated by the number of splenic antibody-forming cells was not significantly different from that of the control (Table 1). Therefore, despite morphological changes in response to different levels of testosterone, the humoral immune response was unaffected under our experimental conditions.

Scent Attractiveness

Two-way ANOVA did not show statistically significant effects of physiological condition of females (oestrous/nonoestrous) and experimental groups of males on the total time that females spent sniffing soiled bedding sampled before and after injection with SRBC (before: $F_{1,250} = 1.25$, $P = 0.26$; after: $F_{3,250} = 1.13$, $P = 0.34$). These times varied from 61.2 ± 2.7 s for samples collected from GDX males to 68.0 ± 3.5 s for samples collected from GDX + 10T males. However, the differences in sniffing of samples collected after and before antigenic challenge (DT) depended on the experimental groups ($F_{3,250} = 2.66$, $P = 0.049$) and not on the physiological condition of females ($F_{1,250} = 0.93$, $P = 0.335$). Injection with SRBC resulted in a significant decline in scent attractiveness in SO and GDX males (Fig. 1). The maximal antigen-induced decline in scent attractiveness (DT) was found in olfactory tests with soiled bedding collected from SO males after and before SRBC administration. DT declined progressively from -10.86 ± 2.94 s in SO males to 1.13 ± 3.21 s in GDX + 10T males.

Urinary Proteins

Two-way ANOVA revealed a statistically significant influence of experimental group (SO, GDX and GDX + 10T) and an interaction between experimental groups and SRBC-treated measures on the concentration of the urinary proteins (group: $F_{2,91} = 5.26$, $P = 0.007$; interaction: $F_{2,91} = 3.71$, $P = 0.028$). Gonadectomy resulted in a decline in urinary protein level in samples collected before injection with SRBC (Fig. 2). Protein level returned to near

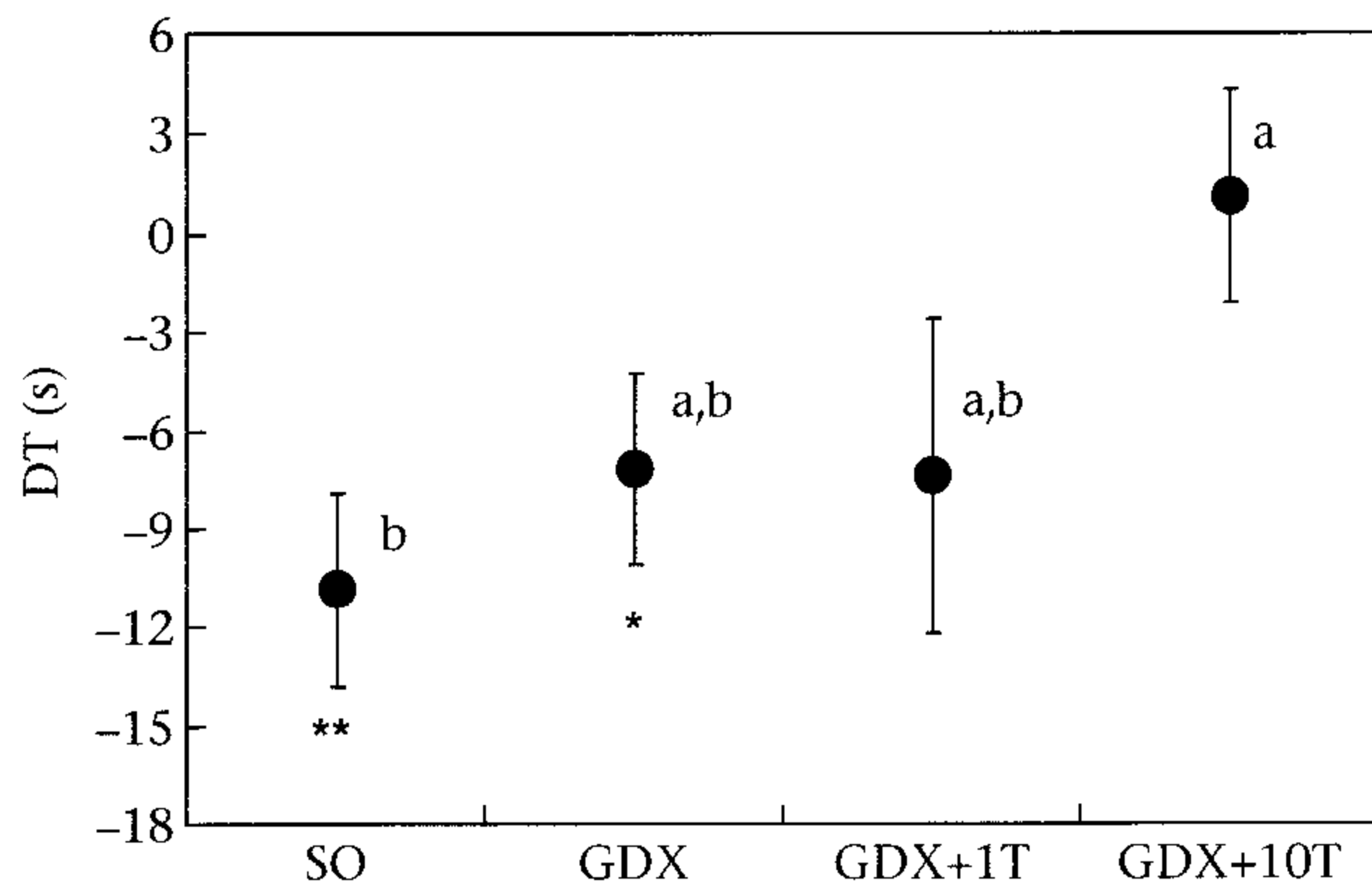


Figure 1. Change in odour attractiveness (DT, $\bar{X} \pm SE$) in sham-operated, gonadectomized and testosterone-treated males after antigenic challenge. DT was calculated as the difference between the times that females investigated male bedding collected after and before the males were injected with SRBC. SO: sham-operated males; GDX: castrated males; GDX + 1T: castrated males injected with 1 mg of Omnadren-250; GDX + 10T: castrated males injected with 10 mg of Omnadren-250. Different letters indicate means that are significantly different (least significant difference test: $P < 0.05$). Asterisks denote statistically significant reduction in sniffing time induced by SRBC treatment ($*P < 0.05$; $**P < 0.01$, paired Student's t test).

control levels in testosterone-treated males of group GDX + 10T. After SRBC administration, SO males showed a significant decrease in urinary proteins in comparison with protein concentration before injection (Fig. 2). Antigenic challenge in GDX and GDX + 10T males did not affect concentrations of the urinary proteins.

Eight fractions of urinary proteins were discriminated by gel filtration chromatography (Fig. 3). Despite the decrease in urinary protein concentration in SO males after SRBC treatment, we could not detect statistically significant differences in the fractional composition of

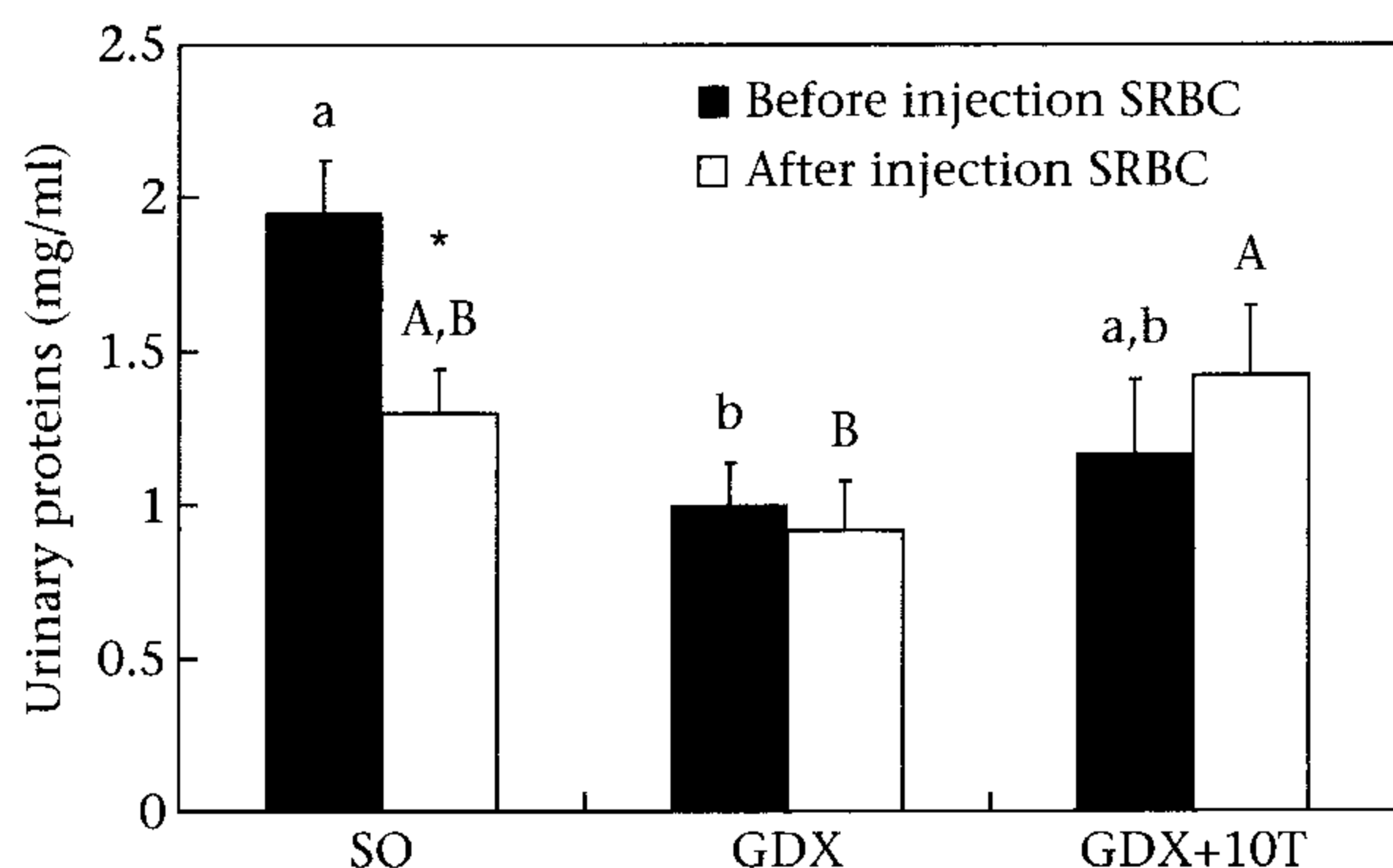


Figure 2. Concentration of urinary proteins in sham-operated, gonadectomized and testosterone-treated males before and after SRBC administration. SO: sham-operated males; GDX: castrated males; GDX + 10T: castrated males injected with 10 mg of testosterone. Values with different letters above columns differ significantly (least significant difference test: $P < 0.05$); a,b for concentration of the urinary proteins before injection with SRBC and A,B after injection. $*P < 0.05$ in comparison with before SRBC treatment for the same experimental group (paired Student's t test).

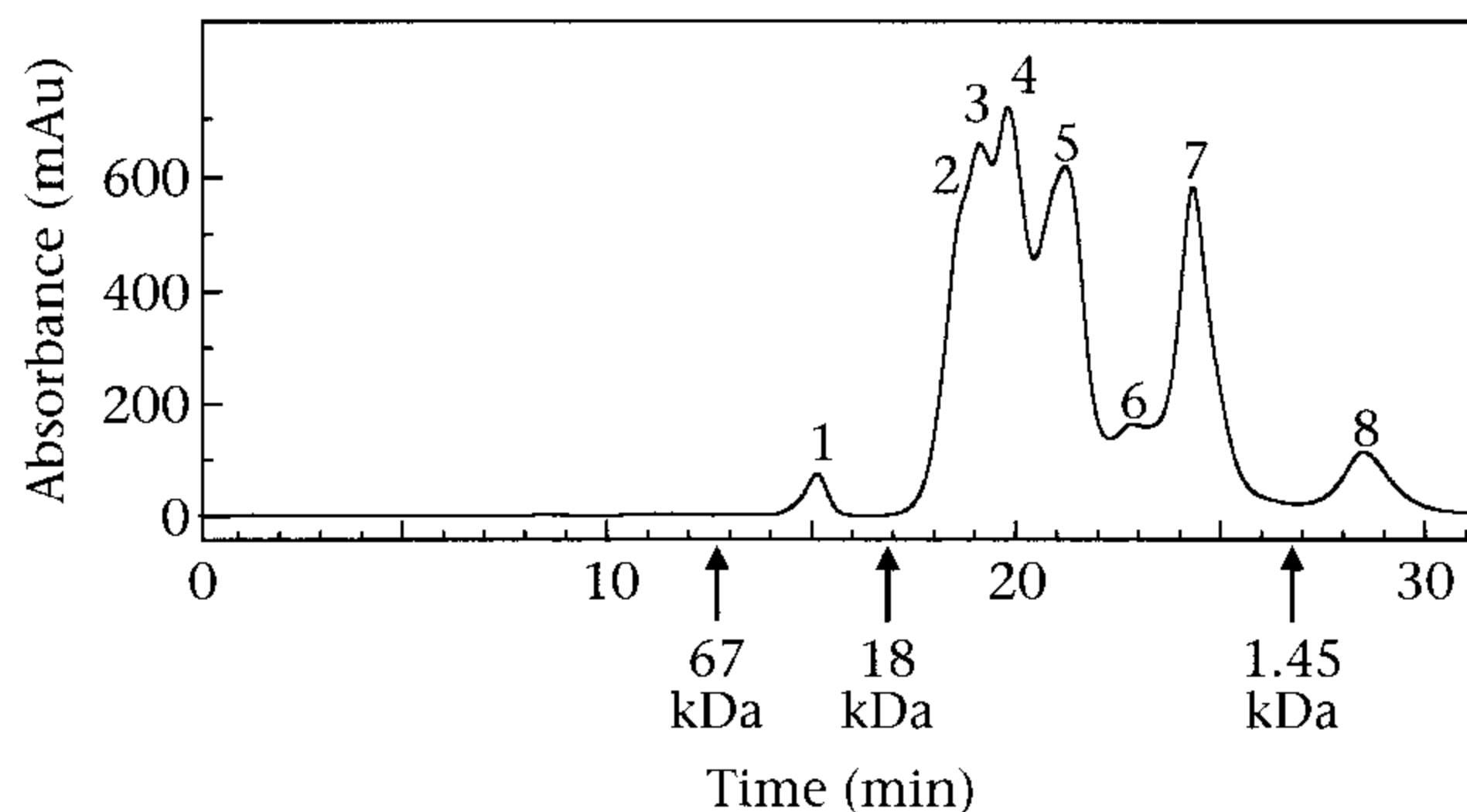


Figure 3. Gel filtration chromatogram of urinary proteins in male mice. Numbers indicate the fractions of proteins.

urinary proteins in samples collected before and after antigenic challenge (Fig. 4). Gonadectomy and testosterone therapy resulted in the same fractional composition of urinary proteins as in SO males. Also as in SO males, SRBC treatment did not affect fractional composition of the urinary proteins in GDX and GDX + 10T males (data not shown).

DISCUSSION

As expected (Moshkin et al. 2000, 2001, 2002), antigenic challenge reduced odour attractiveness in control (SO) male mice. Immune activation by SRBC administration also resulted in a significant drop in urinary protein concentration, supporting a role of these proteins in the decline in scent attractiveness induced by antigenic challenge. According to the literature, urinary protein concentrations of mature males belonging to different strains of mice vary considerably from less than 2 mg/ml up to more than 15 mg/ml (Churakov & Novikov 2000; Marchlewska-Koj et al. 2000; Nevison et al. 2000). Besides strain, the social environment may also influence the excretion of proteins. Social isolation of mature males of the outbred ICR strain results in a significant reduction in urinary protein concentration from 4.78 ± 0.64 mg/ml to 2.29 ± 0.62 mg/ml ($t_{14} = 2,57$, $P = 0.022$) after 2 weeks of being caged singly (E. Y. Kondratjuk, personal commu-

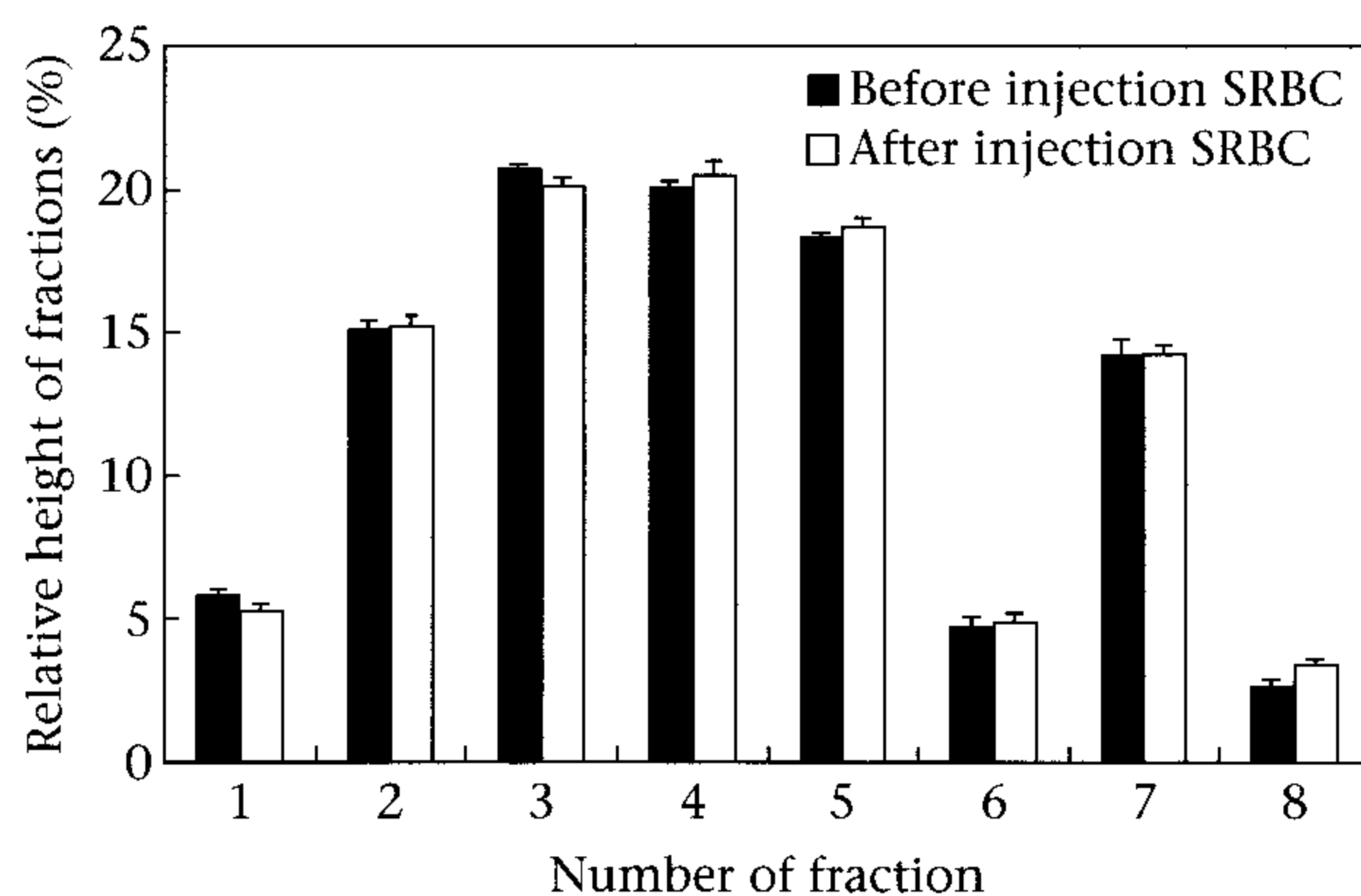


Figure 4. Fractional composition of proteins in urine of sham-operated males before ($N = 15$ samples) and after ($N = 15$ samples) immune response to SRBC. Vertical lines show SEs.

nication). This is similar to the level of urinary proteins we detected in control males that were caged singly for 19 days before sampling.

Furthermore, stabilizing testosterone level by gonadectomy or testosterone administration abolished the decrease in protein concentration in male urine after antigenic challenge. At the same time we could not detect significant differences in the fractional composition of urinary proteins in urine collected before and after SRBC treatment or in urine from gonadectomized and testosterone-treated males.

In contrast to their effect on urinary proteins, gonadectomy and the low dose of androgens did not abolish the differences in time that females spent sniffing soiled bedding sampled from male cages before and after injection with SRBC. Although stabilization of testosterone at the higher level by injection with 10 mg of Omnodren-250 was sufficient to abolish the antigen-induced decline in male attractiveness, our results suggest that the influence of antigenic challenge on chemosignals is only partly caused by a reduction in circulating testosterone and the androgen-dependent concentration of urinary proteins. Among other reasons for the antigen-induced decline in male attractiveness, we cannot exclude the hypothalamic-pituitary-adrenocortical (HPA) system, which is activated after injection with SRBC (Korneva & Shkinek 1988; Moshkin et al. 2002). Hormones of this system secreted in response to stress stimuli can modify chemical signals in male and female mice (Drickamer & McIntosh 1980; Novotny et al. 1986; Cocke et al. 1993; Kavaliers et al. 1998; Weidong et al. 1998), and concentration of plasma corticosterone correlates negatively with odour attractiveness of male mice (Gerlinskaya et al. 1995; Moshkin et al. 2002).

It was surprising, however, here and in our previous paper (Moshkin et al. 2001) that we found equivalent responses in oestrous and nonoestrous female mice regarding the soiled bedding collected before and after SRBC treatment. The physiological condition of females is important for discrimination between male chemical signals in many cases (Mossman & Drickamer 1996; Mucignat-Carreta 2002), but not always. For example, oestrous and nonoestrous females show a similar preference for odour stimuli in such paradigms as adult male versus juvenile male and 'near' male versus 'far' male (Mossman & Drickamer 1996). The comparison of scent attractiveness between uninfected male mice and males infected with the enteric single-host protozoan parasite, *E. vermiformis* showed that females of mixed oestrous-phase can clearly discriminate odour cues of parasitized males (Kavaliers & Colwell 1995a). Furthermore, using odour-induced pain inhibition as an indicator of female aversive responses to chemosignals, Kavaliers et al. (1998) showed that oestrous and nonoestrous females showed similar analgesia in response to odour stimuli of infected or stressed males. Reduced interest in chemosignals of parasitized or antigen-treated males seems to be adaptive for both receptive and nonreceptive females, since it minimizes an infection risk (Kavaliers et al. 1998).

In conclusion, activation of immune defence by injection with foreign antigens reduced both scent

attractiveness and concentration of urinary proteins, probably because of suppression of testosterone secretion (Moshkin et al. 2001, 2002). Stabilization of circulating androgens at low (GDX) or high (GDX + 10T) levels abolished the antigen-induced decline in the urinary proteins. In contrast to proteins, only a high dose of testosterone was sufficient for maintaining scent attractiveness in SRBC-treated males at pretreatment level. This shows that reduction in circulating testosterone and urinary proteins is only part of the SRBC-induced process responsible for the decline in scent attractiveness in antigen-treated males.

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References

- Bacchini, A., Geatani, E. & Cavaggioni, A. 1992. Pheromone binding protein of the mouse *Mus musculus*. *Experientia*, **48**, 419–421.
- Barnard, C. J., Behnke, J. M., Gage, A. R., Brown, H. & Smothurst, P. R. 1998. The role of parasite-induced immunodepression, rank and social environment in the modulation of behaviour and hormone concentration in male laboratory mice. *Proceedings of the Royal Society of London, Series B*, **265**, 693–701.
- Besedovsky, H. & Sorkin, E. 1975. Changes in blood hormone levels during the immune response. *Proceedings of the Society of Experimental Biology and Medicine*, **150**, 466–470.
- Beynon, R. J. & Hurst, J. L. 2003. Multiple roles of major urinary proteins in the house mouse *Mus domesticus*. *Biochemical Society Transactions*, **31**, 142–146.
- Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Annals of Biochemistry*, **72**, 248–254.
- Cavaggioni, A., Sorbi, R. T., Keen, J. N., Pappin, D. J. C. & Findlay, J. C. B. 1987. Homology between the pyrazine-binding protein from nasal mucosa and urinary proteins. *Federation of European Biochemical Societies Letters*, **2**, 225–228.
- Chard, T. 1978. *An Introduction to Radioimmunoassay and Related Techniques*. Amsterdam: North Holland Publishing.
- Churakov, G. A. & Novikov, S. N. 2000. Heterogeneity and differential expression of MUP proteins as a genetic basis of the physiological activity of androgen-dependent pheromones. *Doklady Biological Sciences*, **375**, 549–552. In Russian.
- Cocke, R., Moynihan, J. A., Cohen, N., Grota, L. J. & Ader, R. 1993. Exposure to conspecific alarm chemosignals alters immune responses in BALB/c mice. *Brain, Behavior, and Immunity*, **7**, 36–46.
- Cunningham, A. J. 1965. A method of increased sensitivity for detecting single antibody-forming cells. *Nature*, **207**, 1106–1107.
- Drickamer, L. C. & McIntosh, T. K. 1980. Effects of adrenalectomy on the presence of a maturation delaying pheromone in the urine of female mice. *Hormones and Behavior*, **14**, 146–152.
- Favre, B., Gregoire, A., Preault, M., Ce-zilly, F. & Sorci, G. 2003. Immune activation rapidly mirrored in a secondary sexual trait. *Science*, **300**, 103.
- Ferkin, M. H., Sorokin, E. S., Renfroe, M. W. & Johnston, R. E. 1994. Attractiveness of male odours to females varies directly with

- plasma testosterone concentration in meadow voles. *Physiology and Behavior*, **55**, 347–353.
- Gerlinskaya, L. A., Moshkin, M. P. & Evsikov, V. I.** 1993. The methodical approaches to estimation of stress level in the wild animals. *Ekologiya (Russian Journal of Ecology)*, **1**, 97–100 In Russian.
- Gerlinskaya, L. A., Rogova, O. A., Yakushko, O. F. & Evsikov, V. I.** 1995. Female olfactory choice and its influence on pregnancy in mice. In: *Advances in the Biosciences. Chemical Signals in Vertebrates VII* (Ed. by R. Apfelbach, D. Muller-Schwarze, K. Reutter & E. Weller), pp. 297–302. Oxford: Elsevier.
- Heywood, L. H.** 1980. Testosterone levels in the male laboratory rat: variation under experimental conditions. *International Journal of Andrology*, **3**, 519–529.
- Humphries, R. E., Robertson, D. H. L., Beynon, R. J. & Hurst, J. L.** 1999. Unravelling the chemical basis of competitive scent marking in house mice. *Animal Behaviour*, **58**, 1177–1190.
- Kavaliers, M. & Colwell, D. D.** 1993. Aversive responses of female mice to the odours of parasitized males: neuromodulatory mechanisms and implications for mate choice. *Ethology*, **95**, 206–212.
- Kavaliers, M. & Colwell, D. D.** 1995a. Discrimination by female mice between the odours of parasitized and non-parasitized males. *Proceedings of the Royal Society of London, Series B*, **261**, 31–35.
- Kavaliers, M. & Colwell, D. D.** 1995b. Odours of parasitized males induce aversive response in female mice. *Animal Behaviour*, **50**, 1161–1169.
- Kavaliers, M., Colwell, D. D., Ossenkopp, K.-P. & Perrot-Sinal, T. S.** 1997. Altered responses to female odours in parasitized male mice: neuromodulatory mechanisms and relations to female choice. *Behavioral Ecology and Sociobiology*, **40**, 373–384.
- Kavaliers, M., Colwell, D. D. & Choleris, E.** 1998. Parasitized female mice display reduced aversive responses to the odours of infected males. *Proceedings of the Royal Society of London, Series B*, **265**, 1111–1118.
- Kiesecker, J. M., Skelly, D. K., Beard, K. H. & Preisser, E.** 1999. Behavioural reduction of infection risk. *Proceedings of the National Academy of Sciences, U.S.A.*, **96**, 9165–9168.
- Korneva, E. A. & Shkinek, A. A.** 1978. *Hormones and Immune System*. Leningrad: Nauka In Russian.
- Loehle, C.** 1995. Social barriers to pathogen transmission in wild animal populations. *Ecology*, **76**, 326–335.
- Marchlewska-Koj, A., Cavaggioni, A., Mucignat-Caretta, C. & Olejniczak, P.** 2000. Stimulation of oestrus in female mice by male urinary proteins. *Journal of Chemical Ecology*, **26**, 2355–2366.
- Mashkovskii, M. D.** 1978. *Medicinal Preparations. Handbook for Pharmacotherapy*. Moscow: Medicine In Russian.
- Moshkin, M. P., Dobrotvorsky, A. K., Mak, V. V., Panov, V. V. & Dobrotvorskaya, E. A.** 1998. Variability of immune response to heterologous erythrocytes during population cycles of red (*Clethrionomys rutilus* Pallas) and bank (*C. glareolus* Schreber) voles. *Oikos*, **82**, 131–138.
- Moshkin, M. P., Litvinova, K., Kolosova, I. & Mak, V.** 2000. The changes of odour attraction in male mice treated with non-replicated antigen. *Doklady Russian Academy of Sciences*, **9**, 277–279 In Russian.
- Moshkin, M. P., Kolosova, I., Novikov, Eu. A., Litvinova, E. A., Mershieva, L. V., Mak, V. V. & Petrovskii, D. V.** 2001. Co-modulation of the immune function and the reproductive chemosignals. *Asian–Australasian Journal of Animal Sciences*, **14**, 43–51.
- Moshkin, M. P., Gerlinskaya, L. A., Morozova, O., Bakhvalova, V. & Evsikov, V. I.** 2002. Behaviour, chemosignals and endocrine functions in male mice infected with tick-borne encephalitis virus. *Psychoneuroendocrinology*, **27**, 603–608.
- Moshkin, M. P., Gerlinskaya, L. A. & Evsikov, V. I.** 2003. The role of the immune system in behavioral strategies of reproduction under parasitic pressure. *Journal of General Biology*, **64**, 23–44 In Russian.
- Mossman, C. A. & Drickamer, L. C.** 1996. Odor preferences of female house mice (*Mus domesticus*) in seminatural enclosures. *Journal of Comparative Psychology*, **110**, 131–138.
- Mucignat-Caretta, C.** 2002. Modulation of exploratory behavior in female mice by protein-borne male urinary molecules. *Journal of Chemical Ecology*, **28**, 1853–1863.
- Nelson, J. F., Latham, K. R. & Finch, C. E.** 1975. Plasma testosterone levels in C57Bl/6J male mice: effects of age and disease. *Acta Endocrinologica*, **80**, 744–752.
- Nevison, Ch. M., Barnard, Ch. J., Beynon, R. J. & Hurst, J. L.** 2000. The consequences of inbreeding for recognizing competitors. *Proceedings of the Royal Society of London, Series B*, **267**, 687–694.
- Novotny, M., Jemiolo, B., Harvey, S., Wiesler, D. & Marchlewska-Koj, A.** 1986. Adrenal-mediated endogenous metabolites inhibit puberty in female mice. *Science*, **231**, 722–725.
- Penn, D. J. & Potts, W. K.** 1998. Chemical signals and parasite-mediated sexual selection. *Trends in Ecology and Evolution*, **13**, 391–396.
- Penn, D. J., Schneider, G., White, K., Slev, P. & Potts, W. K.** 1998. Influenza infection neutralizes the attractiveness of male odour to female mice (*Mus musculus*). *Ethology*, **104**, 685–694.
- Robertson, D. H. L. & Beynon, R. J.** 1993. Extraction, characterization, and binding analysis of two pheromonally active proteins of the house mouse (*Mus musculus*). *Journal of Chemical Ecology*, **19**, 1405–1416.
- Rogovin, K., Randall, J. A., Kolosova, I. & Moshkin, M.** 2003. Social correlates of stress in adult males of the great gerbil, *Rhombomys opimus*, in years of high and low population densities. *Hormones and Behavior*, **43**, 132–139.
- Sharrow, S. D., Vaughn, J. L., Zidek, L., Novotny, M. V. & Stone, M. J.** 2002. Pheromone binding by polymorphic mouse major urinary proteins. *Protein Science*, **11**, 2247–2256.
- Singh, P. B.** 2001. Chemosensation and genetic individuality. *Reproduction*, **121**, 529–539.
- Weidong, M., Miao, Z. & Novotny, M. V.** 1998. Role of the adrenal gland and adrenal-mediated chemosignals in suppression of estrus in the house mouse: the Lee-Boot effect revisited. *Biology of Reproduction*, **59**, 1317–1320.
- Willis, C. & Poulin, R.** 2000. Preference of female rats for the odours of non-parasitised males: the smell of good genes. *Folia Parasitologica*, **47**, 6–10.