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Stress Affects Corticosteroid and Immunoglobulin Concentrations in Male House Mice (*Mus musculus*) and Prairie Voles (*Microtus ochrogaster*)

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ABSTRACT. Glucocorticoids, secreted in response to perceived stress, can suppress immunoglobulin (Ig) levels and compromise immune function in mice and rats. Prairie voles (*Microtus ochrogaster*) have been reported to exhibit basal corticosterone concentrations that would cause pathological changes in the immune function of most other rodents. The goals of the present study were to verify that serum corticosterone concentrations are high in prairie voles, as compared with house mice (*Mus musculus*), by measuring serum corticosterone with the same RIA; to examine the effects of mild stressors on corticosterone response in both species and to examine the effects of elevated corticosterone levels on IgM and IgG levels in prairie voles and house mice. After 2 weeks of randomly timed 15-min daily restraint or cold-water swim sessions, animals were injected with sheep red blood cells. The data confirmed that basal blood concentrations of corticosterone were higher in prairie voles than house mice, but these high levels doubled after the first swim session in prairie voles, indicating that the adrenals can respond to stressors by producing increased corticosterone. After stress, antibody production (both IgM and IgG) was reduced in house mice but not in prairie voles, despite higher blood concentrations of glucocorticoids in prairie voles. Although body mass was statistically equivalent between species, prairie voles and mice differed dramatically in adrenal and splenic masses. Average adrenal mass of prairie voles was approximately three times the average mass of these organs in house mice; in contrast, the average splenic mass of house mice was approximately three times that of prairie voles. These data may be relevant to seasonal changes in immune function and survival. COMP BIOCHEM PHYSIOL 118A;3:655–663, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. Glucocorticoids, corticosterone, adrenal, stress, immunoglobulin, immunity, spleen, arvicoline rodents, IgG, IgM

INTRODUCTION

The hormonal stress response has been well characterized among rodents (2). The release of glucocorticoids from the adrenal cortices is a major component of the cascade of physiological modifications elicited in response to a perceived stressor. Glucocorticoids affect metabolic, endocrine, neural and immune functions [reviewed in (42)]. Typically, activation of the hypothalamic-pituitary-adrenal (HPA) axis mobilizes energy and suppresses various anabolic processes (11). Although the secretion of corticosteroids may be adaptive during acute physical stress, prolonged secretion and elevated blood levels of glucocorticoids have been associated with the development of pathological conditions, including sustained immunocompromise (20,30).

Corticosterone is the primary glucocorticoid produced in rodents. This steroid is secreted in a circadian pattern with highest concentrations reported before or during the onset of daily activity (11). Corticosterone levels reported for *Microtus* species range from 3 to 10 times those of laboratory strains of mice and rats (14,27,37,43,46). Prairie voles (*Microtus ochrogaster*) exhibit the highest corticosterone concentrations within the *Microtus* genus of arvicoline rodents.

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Corticosterone levels in this species average approximately 1000 ng/ml at the circadian peak (46). In contrast to voles, average peak blood corticosterone levels in mice have been reported to range between approximately 100 and 300 ng/ml (39). However, prairie voles do not exhibit any of the obvious deleterious consequences usually associated with high blood levels of glucocorticoids that have been reported for other rodent species [e.g., (11)], and the results of dexamethasone suppression tests suggest that prairie voles are partially glucocorticoid resistant (46). The nature and extent of the adaptation to high circulating corticosterone levels remain unspecified.

Adrenocortical hormones suppress immune function in both humans and nonhuman animals [reviewed in (1, 16,20)]. Glucocorticoids, released in response to stressful stimuli, can suppress T-cell activity (10,22,24). Stressful stimuli, such as 2-hr restraint of mice, have also been shown to suppress antibody production (4,5,19,44). Adrenalectomy reverses the effects of stress on immune function (3,12). The precise mechanisms by which the HPA axis affects the immune system are unknown, but the mechanisms probably involve cytokine release rates from activated immunological cells (3,20,44).

The purpose of the present study was to compare the effects of mild stressors on corticosterone secretion in house mice (*Mus musculus*) and prairie voles (*M. ochrogaster*). We also compared the effects of mild stress on IgM and IgG responses after injection with sheep red blood cells (SRBC) in both species.

MATERIALS AND METHODS

Experiment 1: Restraint Stress

ANIMALS. Thirty adult (>60 days of age) male CF-1 house mice (*M. musculus*) were purchased from Charles River (Boston, MA). Thirty adult (>60 days of age) male prairie voles (*M. ochrogaster ochrogaster*) were obtained from breeding pairs established in our laboratory from descendants of animals trapped near Urbana, Illinois.

HOUSING CONDITIONS. The voles were weaned at 21 days of age and housed with same-sex siblings. Three weeks before the initiation of the experiment, siblings were separated and singly housed. Mice were singly housed, from their arrival, throughout the study. Food (Agway Prolab 1000, Syracuse, NY) and tap water were available at all times before and during the study. All animals were housed individually in polypropylene cages (27.8 × 7.5 × 13 cm) in colony rooms with 24-h-16L:8D cycles (lights illuminated at 0700 hr Eastern Standard Time) at 21 ± 2°C with 50% ± 5% relative humidity.

EXPERIMENTAL PROTOCOL. Three blood samples were obtained from the retro-orbital sinus of the eye (40). A baseline sample was obtained 10 days before the start of experimental manipulations. A second sample was obtained immediately after 15 min of restraint in a Plexiglass injection tube (length 10.5 cm; radius 1.75 cm), and a final sample was obtained after 17 days of daily 15-min bouts of restraint. Experimental animals were restrained for 15 min between 1100 and 1600 hr. Immediately after the restraint, the animals were lightly anesthetized with methoxyflurane vapors (Metofane; Pitman-Moore, Mundelein, IL) and a blood sample was obtained within 2 min of release from the restraint tube. Control animals remained in their home cage.

AUTOPSY. Body mass was recorded before autopsy. After collection of the final blood sample, animals were killed via cervical dislocation under methoxyflurane anesthesia; spleens and adrenal glands were removed and weighed. Absolute and relative organ masses were recorded.

SERUM CORTICOSTERONE ASSAY. Corticosterone was assayed by radioimmunoassay (RIA) using the ICN Biomedicals, Inc. (Costa Mesa, CA) ^{125}I kit. The mouse serum dilution was prepared according to the guidelines furnished by ICN; the same procedures were followed for the prairie vole except that the vole serum was diluted 1:2121. This RIA has been completely validated for use in prairie voles (14,46). The corticosterone assay was highly specific, cross-reacting at less than 0.3% with other steroid hormones. Serum corticosterone values were determined in a single RIA.

STATISTICAL ANALYSES. Data for body and organ masses were analyzed using two-way ANOVA (Systat, Inc., Evanston, IL). Corticosterone, IgM and IgG data were analyzed using mixed-design ANOVA (Systat, Inc.). All analyses were followed by planned comparisons of pairwise means. Differences between group means were considered statistically significant if $P < 0.05$.

Experiment 2: Swim Stress

ANIMALS AND HOUSING CONDITIONS. Thirty adult (>60 days of age) male CF-1 house mice (*M. musculus*) were purchased from Charles River. Thirty adult (>60 days of age) male prairie voles (*M. ochrogaster*) were obtained from breeding pairs established in our laboratory from descendants of animals trapped near Urbana, Illinois. Voles were weaned at 21 days of age and housed separately before the onset of the study.

EXPERIMENTAL PROTOCOL. Four blood samples were drawn from each animal over the course of the experiment. On the first day of the experiment, animals were lightly anesthetized with methoxyflurane vapors (Metofane; Mallinckrodt Veterinary, Mundelein, IL), and a baseline blood sample was obtained via the retro-orbital sinus (40).

Experimental animals were subjected to a daily 3-min cold water (15°C) swim. The swimming trials began on the 15th day and continued until the end of the experiment on day 29. Animals were transported in their cages from the

colony room to an isolated adjoining room where the swimming trials took place. They were individually placed into a polypropylene cage (45 × 24 × 20 cm) filled with water to an approximate height of 15 cm. The mice and voles were subjected to the swim procedure at random times and random order during the daily light phase. To minimize variation in handling the animals, experimenters wore fresh latex gloves and used new plastic cups to transfer each of the animals between their cages and the water. After each swim trial animals were placed back in their cages with 5 cm^3 of cotton batting and left under a lamp for 1 min to assist with drying before returning to the colony. Control animals were moved to the swim room, given an equal amount of lamp time and cotton batting in their cages before returning to the colony room.

A second blood sample was obtained on day 15 from lightly anesthetized animals. On day 19, all animals received an intraperitoneal injection of 0.2 ml of a 1% suspension of SRBC (approximately 3000 cells). A third blood sample was collected on day 22 to assess antibody concentrations 3 days post-injection of SRBC. A final blood sample was taken on day 29 at autopsy to assess antibody concentrations 10 days post-injection of SRBC. The second, third and fourth blood samples were obtained immediately after swim trials for the experimental animals.

All blood samples were stored for 1 hr at room temperature after collection. After clots were removed, samples were centrifuged at 4°C for 1 hr at 3500 rpm, the serum aspirated from the microtube and stored at −80°C.

AUTOPSY AND CORTICOSTERONE ASSAY. Autopsies and corticosterone assays were conducted as in experiment 1. Baseline and the third and fourth blood samples were also assayed for IgG and IgM levels.

PREPARATION OF SRBC AND ANTIBODY ASSAY. Three milliliters of SRBC (Truslow Farms, Chestertown, MD) were washed three times in 0.2 M phosphate-buffered saline (PBS, pH 7.2). SRBC were then resuspended in PBS for a final concentration of 0.01%.

IgG and IgM levels in the blood were determined using separate sandwich ELISAs previously developed for house mice and validated for use with prairie voles in our laboratory. Serial dilutions yielded values in parallel to the standard curves. Goat polyclonal antibodies raised against mouse IgG and IgM (Cappel) were diluted 1:3000 in a carbonate/bicarbonate buffer (0.1 M, pH 9.6). A total of 100 μl per well of each antibody was added to separate 96-well immunoplates (Nunc, Maxisorp) and the plates incubated overnight at 4°C. The following day, the plates were washed four times with wash buffer (PBS [0.05 mM pH 7.4] containing 0.05% Tween-20 and 0.001% NaN_3) using an automatic microplate washer (BioRad, Model 1550). A standard curve (upper limit 1000 μg/ml; lower limit 0.001 μg/ml) was prepared using purified mouse IgG or IgM (Sigma) diluted in standard diluent (PBS [0.05 M, pH 7.4] containing 0.05% Tween-20). The standards (100 μl per well in triplicate) and samples of mouse and vole serum (100 μl per well in duplicate diluted 1:100 with standard diluent) were placed in wells on the plates. The plates incubated overnight at 4°C. The following day the plates were washed four times, and 100 μl of alkaline phosphatase-conjugated sheep-anti-mouse IgG or IgM (diluted 1:2500 in standard diluent) were added to each well. The plates incubated overnight at 4°C. The following day the plates were washed and 100 μl of substrate buffer (0.1 mM *p*-nitrophenyl phosphate in diethanolamine buffer [0.1 M, pH 9.5] containing 5 mM $MgCl_2$) were added to each well. The plates were incubated for 30 min and the optical density of the resulting colored product in each well was measured at 490 nm using a microplate reader (Bio-Rad, Model 450). The absolute concentrations of IgG or IgM in the samples were determined relative to the standard curve.

RESULTS

Experiment 1: Restraint Stress

Although prairie voles and house mice did not differ in body mass ($P > 0.05$) (Table 1), prairie voles had significantly

TABLE 1. Mean ± SEM body mass (g) and relative organ masses (mg/g body mass) in restrained and control animals

	Body mass (g)	Paired adrenal mass (mg/g)	Splenic mass (mg/g)
Prairie voles			
Control	35.79 ± 1.79 (n = 13)	0.339 ± 0.041^{ac} (n = 13)	0.81 ± 0.07^{eg} (n = 13)
Restrained	35.83 ± 1.11 (n = 13)	0.437 ± 0.092^{bd} (n = 13)	0.83 ± 0.07^{fh} (n = 13)
House mice			
Control	34.92 ± 0.57 (n = 14)	0.123 ± 0.068^{cd} (n = 14)	3.27 ± 0.12^{ef} (n = 14)
Restrained	31.91 ± 0.53 (n = 14)	0.139 ± 0.013^{ab} (n = 14)	3.04 ± 0.19^{gh} (n = 14)

Groups sharing the same letter are statistically different.

heavier adrenals (adjusted for body mass) compared with mice ($F_{1,48} = 28.864$, $P < 0.01$) (Fig. 1 and Table 1). In contrast, both absolute and relative splenic masses were higher in house mice than prairie voles ($P < 0.01$ for both absolute and relative masses) (Fig. 1 and Table 1). Stress did not affect body mass ($P > 0.05$), paired adrenal mass ($P > 0.05$) or splenic mass in either species ($P > 0.05$) (Fig. 1 and Table 1).

Baseline serum corticosterone levels were significantly elevated among prairie voles as compared with house mice across groups ($F_{1,22} = 107.264$, $P < 0.01$); however, exposure to stress did not affect corticosterone levels within either species ($P > 0.05$) (Table 2). Corticosterone levels also differed significantly across repeated blood samples in house mice ($F_{2,16} = 6.567$, baseline values compared with sample 3 values; $P < 0.05$) but not prairie voles ($P > 0.05$) (Table 2). In house mice, corticosterone levels were significantly elevated after chronic restraint stress (i.e., after multiple restraint sessions) compared with acute restraint stress (i.e., immediately after the first restraint session) ($t_{16} = -2.338$, $P < 0.05$) (Table 2). All other relevant pairwise comparisons of mean differences were not statistically significant.

Experiment 2: Swim Stress

Again, body mass did not differ significantly between species ($P > 0.05$) (Table 3). Prairie voles had significantly heavier adrenals (both absolute and adjusted for body mass) compared with mice ($F_{1,38} = 69.37$, $P < 0.01$) (Fig. 2 and Table 3). Conversely, both absolute and relative spleen mass were significantly heavier in mice than in voles ($F_{1,42} = 71.26$, $P < 0.01$ in both cases) (Fig. 2 and Table 3). No significant differences existed in body, paired adrenal or splenic mass between stressed and control animals in either species ($P > 0.05$) (Fig. 2 and Table 3).

Serum corticosterone levels differed significantly across repeated blood samples in prairie voles ($F_{3,42} = 4.83$, $P < 0.01$) but not mice ($P > 0.05$) (Table 4). In prairie voles, baseline serum corticosterone concentrations more than doubled after their first swim session ($t_{11} = -4.49$, $P < 0.05$). Blood levels of corticosterone in prairie voles were lower after multiple swim sessions as compared with corticosterone levels measured immediately after the initial swim session ($t_{11} = 3.57$, $P < 0.01$) (Table 4). In contrast, circulating corticosterone levels were significantly elevated from baseline levels after the first swim session among house mice (sample 2 vs sample 1) ($t_6 = -3.18$, $P < 0.05$) and after multiple swim sessions (i.e., sample 4 vs sample 1) ($t_7 = -3.49$, $P < 0.05$) (Table 4). For prairie voles in the control group, corticosterone levels measured in sample 4 were significantly higher than sample 2 ($t_5 = -2.97$, $P < 0.05$), despite the absence of swim sessions (Table 4). All other pairwise comparisons of mean differences were not statistically significant ($P > 0.05$ in each case).

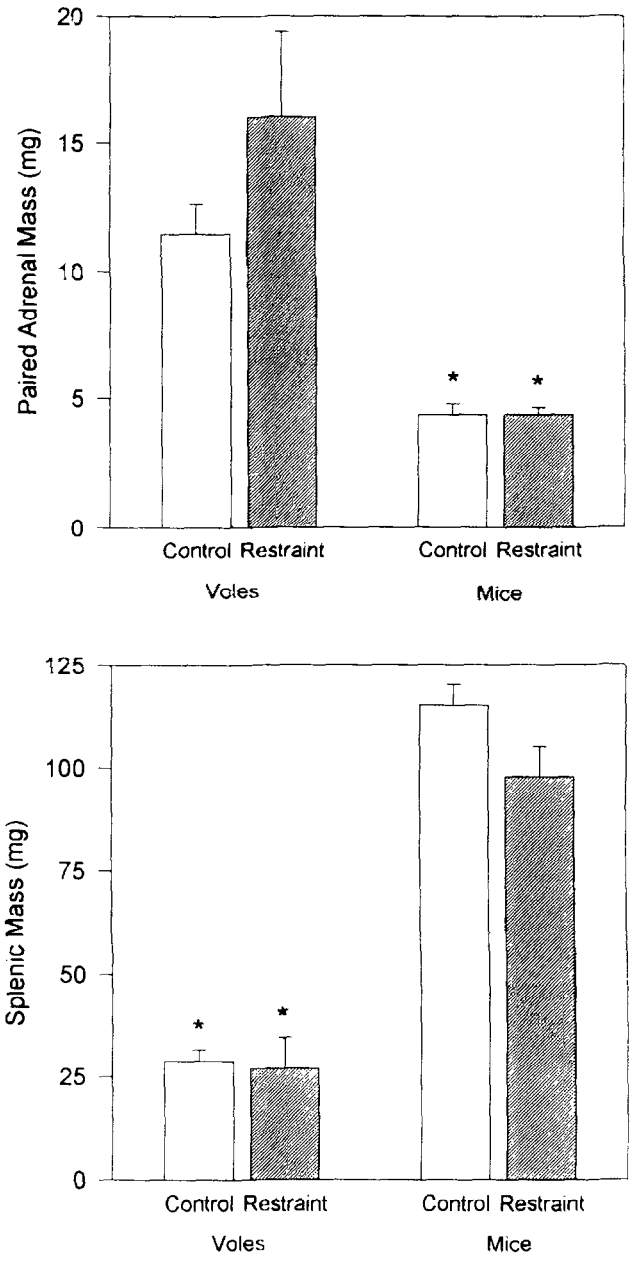

FIG. 1. (Top) Mean ± SEM paired adrenal masses (mg) for prairie voles and house mice that were either maintained in their cages, except for blood sampling (Control), or subjected to 15 min of daily restraint in a Plexiglass injection tube for 17 consecutive days (Restraint). (Bottom) Mean ± SEM splenic masses (mg) for prairie voles and house mice that were either maintained in their cages, except for blood sampling (Control), or subjected to 15 min of daily restraint in a Plexiglass injection tube for 17 consecutive days (Restraint).

Serum IgG levels were significantly higher in mice than in prairie voles ($F_{1,19} = 22.04$, $P < 0.01$) (Table 5). Swim

TABLE 2. Mean ± SEM serum corticosterone levels (ng/ml) in restrained and control animals

	Sample 1 (Day −10)	Sample 2 (Day 0)	Sample 3 (Day 17)
Prairie voles			
Control	1435.0 ± 261.8[ac] (n = 7)	1351.0 ± 272.7 (n = 8)	1840.7 ± 254.4[ei] (n = 12)
Restrained	1330.0 ± 294.9[b] (n = 7)	1425.0 ± 316.9 (n = 13)	1158.6 ± 227.4[j] (n = 13)
House mice			
Control	247.8 ± 40.4[abd] (n = 10)	143.0 ± 26.7 (n = 14)	1079.4 ± 156.2[de] (n = 14)
Restrained	195.3 ± 39.8[bcf] (n = 11)	160.1 ± 41.1 (n = 12)	405.0 ± 83.4[fj] (n = 13)

Letters and conventions as in Table 1.

stress did not significantly affect IgG levels between stressed and control animals in either species (overall ANOVA; $P > 0.05$). However, IgG levels differed significantly across repeated blood samples in mice ($F_{1,11} = 4.34$, $P < 0.05$) but not in voles ($P > 0.05$). Mice subjected to multiple swim sessions had lower IgG levels compared with levels of animals measured after the first swim session ($t_{10} = 2.64$, $P < 0.05$) (Table 5). All other pairwise comparisons of mean differences were not statistically significant.

Baseline serum IgM values were statistically equivalent across all groups, except that the basal IgM levels of prairie voles that were assigned to the swim group were significantly lower than animals in the other experimental groups. Control voles, but not prairie voles undergoing swim sessions, displayed a significant reduction ($P < 0.05$) in serum IgM levels 3 days after SRBC injection. Serum IgM levels did not change among prairie voles undergoing cold water swim after SRBC treatment ($P > 0.05$) (Table 6). In contrast, serum IgM levels of house mice dramatically rose 3 days after SRBC treatment ($P < 0.001$) (Table 6) but returned to basal levels by day 10 ($P > 0.05$) (Table 6). There was no difference in IgM concentration between control mice and mice that were subjected to 15-min daily swim sessions ($P > 0.05$) (Table 6).

DISCUSSION

The results confirmed and extended previous studies that reported that basal blood concentrations of corticosterone were elevated in prairie voles as compared with house mice (7,14,46). Neither prairie voles nor house mice displayed changes in blood levels of corticosterone after the restraint sessions, suggesting that this manipulation was not perceived as stressful. In contrast, blood corticosterone levels increased significantly from basal levels in both species immediately after the first swim session. In house mice, blood corticosterone levels remained elevated after repeated swim sessions; in prairie voles, however, blood levels of corticosterone significantly decreased after 1 week and then rebounded to basal levels after 2 weeks of swim sessions. Basal serum IgG, but not IgM, levels were higher in house mice than in prairie voles. Repeated swim stress did not affect IgG levels in voles but reduced serum IgG levels in house mice. IgM levels did not change in prairie voles after SRBC

TABLE 3. Mean ± SEM body mass (g) and relative organ masses (mg/g body mass) of control animals and animals subjected to daily 15-min swim sessions

	Body mass (g)	Paired adrenal mass (mg/g)	Splenic mass (mg/g)
Prairie voles			
Control	39.49 ± 2.01 (n = 12)	0.289 ± 0.033[ad] (n = 8)	2.61 ± 0.30 (n = 8)
Swim	37.62 ± 1.43 (n = 12)	0.346 ± 0.037[bc] (n = 12)	1.23 ± 0.13[ef] (n = 12)
House mice			
Control	38.39 ± 0.62 (n = 12)	0.089 ± 0.011[ac] (n = 10)	2.47 ± 0.13[e] (n = 12)
Swim	39.70 ± 0.80 (n = 12)	0.105 ± 0.014[bd] (n = 12)	2.65 ± 0.16[f] (n = 12)

Letters and conventions as in Table 1.

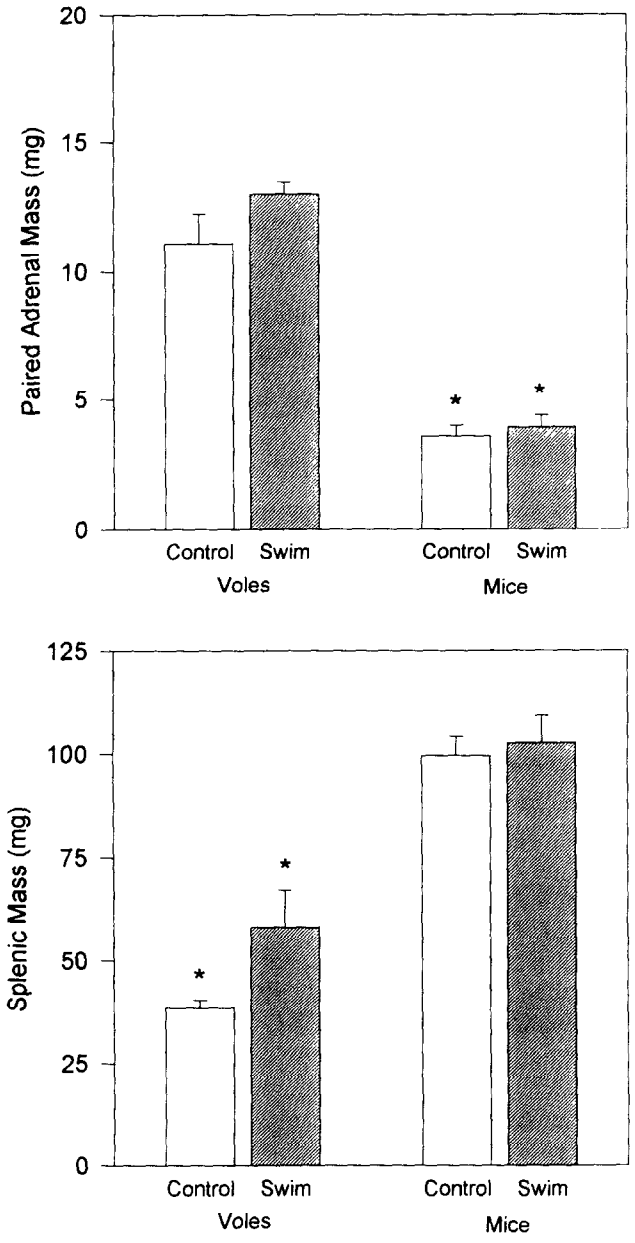

FIG. 2. (Top) Mean ± SEM paired adrenal masses (mg) for prairie voles and house mice that were transported to the swim room but did not swim (Control) or were subjected to 15 min of daily swim sessions (Swim). (Bottom) Mean ± SEM splenic masses (mg) for prairie voles and house mice that were transported to the swim room but did not swim (Control) or were subjected to 15 min of daily swim sessions (Swim).

treatment but rose within 3 days in house mice injected with SRBC; there were no significant differences between control animals and animals subjected to swim sessions. Despite similar body mass among prairie voles and house mice, the two species differed dramatically in adrenal and spleen masses. The average adrenal mass of prairie voles was approximately three times that of house mice. In contrast, the average splenic mass of house mice was approximately three times that of prairie voles. Taken together, the serum corticosterone and adrenal mass data indicate that prairie voles secrete much higher levels of glucocorticoids than house mice. Although immune function was not tested directly, the IgM, IgG and spleen mass data suggest that immune function may be reduced in prairie voles as compared with house mice [cf. (21)].

Profound stress (i.e., restraint for 12 hr/day) compromised immune function [e.g., (35,36)]. The goal of the present study was to assess the effects of *mild* stressors on corticosterone and immunoglobulin levels. Neither prairie voles nor mice appeared to perceive the 15 min of restraint to be more stressful than the multiple blood sampling. Serum corticosterone levels did not differ from baseline levels after restraint in prairie voles; however, repeated episodes of restraint led to elevated serum corticosterone levels in house mice. Because individual prairie voles slept in the restraint devices on several occasions, swimming was used as a stressor in experiment 2. Cold water swim evokes HPA axis activation and subsequent immunocompromise in mice (17). Both species demonstrated elevated corticosterone levels immediately after swimming, but house mice appeared to adapt to the swimming; day 29 corticosterone levels returned to baseline levels in mice. Prairie voles did not show this adaptation. These results indicate that the high corticosterone levels in prairie voles are not asymptotic and do not reflect the effects of captivity induced on the HPA axis.

Because of the small blood samples obtainable from these rodents during sequential bleeding, it was not possible to discriminate the amount of bound vs free glucocorticoids in addition to conducting the immunological assays. Therefore, total (bound + free) levels of circulating corticosterone are presented. In an independent study, it was determined that prairie voles exhibit free corticosterone levels two to three times higher than in laboratory strains of rats and mice (46).

Stressors as diverse as inescapable electric shock, swimming, defeat in an agonistic encounter and conspecific alarm chemosignals elevate corticosterone levels and suppress antibody production in house mice (15,17,22,50). IgM levels typically peak 3–5 days after treatment with SRBC; IgG levels begin to increase about 7 days post-injection and reach peak values about 10–14 days after SRBC injection (47). Neither IgM nor IgG levels were changed after prairie voles were injected with SRBC. These results suggest that circulating levels of immunoglobulins of prairie voles may be more buffered from HPA activation than in house mice. In our study, both serum IgM and IgG levels were elevated in house mice after their first swim session but reduced after long-term swim sessions. Typically, only IgM should have been elevated at day 3 and only IgG should have been elevated 10 days after SRBC injection. This discrepancy may reflect the disparate response to stressors of different magnitude. Moderate to severe stressors suppress immune func-

TABLE 4. Mean ± SEM serum corticosterone levels (ng/ml) in control animals and animals subjected to daily 15-min swim sessions

	Sample 1 (Day 10) Baseline	Sample 2 (Day 15) Day 3 post-SRBC	Sample 3 (Day 22) Day 10 post-SRBC	Sample 4 (Day 29)
Prairie voles				
Control	890.17 ± 138.72^{abc} (n = 5)	1807.70 ± 440.59^{ad} (n = 8)	2206.56 ± 503.32^{b} (n = 8)	2242.76 ± 409.40^{cd} (n = 6)
Restrained	1044.21 ± 236.01^{e} (n = 9)	2537.97 ± 331.55^{ef} (n = 12)	1465.10 ± 224.18^{f} (n = 11)	2319.90 ± 329.93 (n = 12)
House mice				
Control	237.88 ± 84.86 (n = 7)	191.59 ± 46.58^{i} (n = 12)	318.17 ± 42.12^{j} (n = 12)	237.15 ± 41.53^{k} (n = 12)
Restrained	306.75 ± 220.70 (n = 5)	465.52 ± 78.68^{i} (n = 12)	465.52 ± 78.68^{j} (n = 12)	514.93 ± 107.03^{hk} (n = 9)

Letters and conventions as in Table 1.

TABLE 5. Mean ± SEM serum IgG levels (μg/ml) in control animals and animals subjected to cold water swim

	Sample 1 (Day 0) Baseline	Sample 2 (Day 22) Day 3 post-SRBC	Sample 3 (Day 29) Day 10 post-SRBC
Prairie voles			
Control	1.56 ± 0.60^{ac} (n = 12)	2.31 ± 0.84 (n = 13)	2.61 ± 0.30 (n = 8)
Swim	2.93 ± 1.24^{bd} (n = 6)	3.92 ± 1.22 (n = 6)	1.23 ± 0.13 (n = 12)
House mice			
Control	8.22 ± 1.69^{ab} (n = 7)	10.10 ± 1.85 (n = 10)	10.84 ± 1.63 (n = 12)
Swim	11.79 ± 5.33^{cd} (n = 6)	17.73 ± 0.36^{e} (n = 12)	8.11 ± 1.61^{e} (n = 10)

Letters and conventions as in Table 1.

TABLE 6. Mean ± SEM serum IgG levels (μg/ml) in control animals and animals subjected to cold water swim

	Sample 1 (Day 0) Baseline	Sample 2 (Day 22) Day 3 post-SRBC	Sample 3 (Day 29) Day 10 post-SRBC
Prairie voles			
Control	2.17 ± 1.61^{ad} (n = 12)	0.40 ± 0.03^{d} (n = 13)	0.55 ± 0.06 (n = 12)
Swim	0.40 ± 0.17^{abc} (n = 6)	0.36 ± 0.39 (n = 6)	0.37 ± 0.04 (n = 6)
House mice			
Control	1.48 ± 0.13^{be} (n = 7)	12.81 ± 0.56^{ef} (n = 10)	1.47 ± 0.54^{f} (n = 12)
Swim	2.51 ± 1.92^{cg} (n = 6)	12.23 ± 0.46^{gh} (n = 12)	1.68 ± 0.62^{h} (n = 10)

Letters and conventions as in Table 1.

tion; mild stressors, such as our swim stress protocol, enhance immune function (i.e., IgG secretion) and clinical outcome in mice (38).

Rapid changes in glucocorticoid levels have been implicated in several dramatic seasonal cycles in mortality. For instance, the brown marsupial mouse (*Antechinus stuartii*) has a highly synchronized breeding season that is followed in the field by the death of all reproductive males (48,49) and in the laboratory by mortality or reproductive senescence (49). Death results from hyperactivity of the adrenal glands, apparently induced by the stress of breeding and the onset of several opportunistic diseases, cancer and hemorrhagic ulceration of the intestines reflecting profound immunocompromise (7,25,26,48). The effects of increased adrenal activity on immune function and survival has been hypothesized to account for the large population crashes observed among cycling arvicoline rodent populations [e.g., (8,28)]. Our study leaves open the question of the effects of moderate stress on immune function in voles, but certainly peak population densities could increase adrenal activation that could lead to suppressed immune function (28). It is worth noting, however, that in the present study, voles displayed little change in IgM or IgG levels in response to repeated exposures to stress. This could reflect lower sensitivity or specificity in the ELISA for mouse IgG and IgM as compared with vole IgG and IgM, but the ELISA was validated previously for prairie voles. The differences in splenic mass also suggest that immune function may be reduced in prairie voles relative to house mice. However, the lack of effect of the swim sessions and elevated corticosterone levels on IgG and IgM levels might again reflect a better buffering of prairie vole immune function from the suppressive effects of high corticosterone levels on IgG and IgM concentrations than house mice. This is reasonable given the high levels of circulating corticosterone levels in prairie voles as compared with house mice and consistent with the hypothesis that this species may be glucocorticoid resistant (21,46).

The organ mass differences between species do not reflect the stressors of captivity on voles. Snap-trapped prairie voles exhibited adrenal and splenic masses similar to the values in the present study (8). Although adrenal weight is an unreliable index of adrenocortical activity [reviewed in (23)], there is a positive correlation between relative adrenal mass and adrenocortical secretion in voles (9). Adrenal and splenic weights vary seasonally among arvicoline rodents, and seasonal changes in immune function, infection and mortality have also been reported (8,45). Arvicoline rodents are well-known for their dramatic fluctuations in population density. Population crashes, usually coincident with bountiful resources in spring, have been attributed to both intrinsic and extrinsic factors. Increased HPA axis activation in response to crowding (8) and subsequent immune dysfunction (28) may be involved in population crashes. Seasonal changes in adrenal function may drive seasonal changes in immune function. Photoperiod, a reliable cue about time of year, affects adrenal mass in arvicoline rodents [e.g., (45)]. Photoperiod also directly affects immune function in rodents (6,32,33). Reductions in food quantity and quality and low ambient temperatures increase HPA activities and impair synthesis of antibodies (13, 18,29,31). The elevated corticosterone levels observed among captive prairie voles may reflect the stressor of "chemosensory" crowding in the animal colony [cf., (34)]. Further studies are required to determine if olfactory-isolated voles also exhibit elevated glucocorticoid levels. Additional studies are also needed in the field to understand the effects of naturalistic stressors on seasonal changes in immune function and mortality in arvicoline rodents and to determine if the high glucocorticoid levels in these species contribute to their reduced longevity.

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