Voluntary physical activity prevents stress-induced behavioral depression and anti-KLH antibody suppression

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Moraska, Albert, and Monika Fleshner. Voluntary physical activity prevents stress-induced behavioral depression and anti-KLH antibody suppression. Am J Physiol Regulatory Integrative Comp Physiol 281: R484–R489, 2001.—The current study addressed whether physical activity can buffer stress-induced “behavioral depression” and immunosuppression. Adult, male Sprague-Dawley rats were housed with either a mobile (physically active) or immobile (sedentary) running wheel and exposed to either stress (in-escapable tail shock) or no stress (home cage control). Voluntary wheel running began 4 wk before stressor exposure. Immediately before stress, all rats were administered an intraperitoneal injection of keyhole limpet hemocyanin (KLH; 200 μg), and anti-KLH Ig was measured weekly for 4 wk using ELISA. Prior physical activity reduced the stress-induced behavioral depression and prevented the stress-induced suppression of anti-KLH IgM and IgG2a antibodies. Anti-KLH IgG1 was stress insensitive. These data suggest that physical activity can buffer the negative impact of stress on behavior and acquired immune function.

keyhole limpet hemocyanin; exercise; wheel running

EXPOSURE TO MENTAL OR PHYSICAL stressors can produce “behavioral depression” and immunosuppression. For example, Maier et al. (26) reported that exposure to inescapable tail-shock stress produces a reduction in spontaneous wheel-running behavior for several weeks. This reduction in behavior or behavioral depression was both prevented and reversed by administration of antidepressant medications. Stress exposure can also result in immunosuppression. For example, exposure to both acute (19) and chronic (15) restraint, as well as acute electric tail shock (17), rotational stress (12), and chronic forced physical activity (27), all have resulted in a suppression of the antibody response to antigen. Stress-associated reductions in antibody have been reported using several types of antigen including replicating virus (34) and benign protein, i.e., keyhole limpet hemocyanin (KLH) (17). The effect of stress on reduction of specific antibody could have health implications. For example, a decrease in antibody to herpes simplex virus could result in an increase in viral replication, which renders the organism more susceptible to disease caused by that virus (24).

In contrast to stress, physical activity elicits feelings of health and vigor in humans. Moderate physical activity reduces cardiovascular disease (28), improves blood lipid profiles (35), and may act as an antidepressant (8). In addition, moderate physical activity can have beneficial effects on immune-related health and has been proposed as a modality for alleviation of emotional stress (11). For example, individuals who were physically active had no increase in the incidence of illness during times of high stress (2). The role physical activity has on acquired immune function is difficult to study in humans. Human studies typically involve assessment of in vitro peripheral blood lymphocytes and, therefore, may lead to incomplete assessment of immune function (5). Physical activity paradigms using animals can provide insight into the effects of activity on health status and specific immune function due to better access to immune tissues or choice of antigen to administer.

In animal studies, physical activity has resulted in both positive and negative health outcomes. For example, survival of mice infected with Salmonella typhimurium was significantly increased in animals that had access to voluntary activity wheels for 16 days before infection (4). One explanation for the protective effect may be an increased antibody titer. Liu and Wang (25) found increased serum antibody against S. typhimurium in mice that had been physically active before inoculation (25). However, another survival study initiated swim activity in mice simultaneously with coxsackievirus B3 infection (3). Mice that were infected with virus at the time of activity onset exhibited significantly decreased survival rates. Thus physical activity before infection may influence animal survivability, and one mechanism of this protection may be the antibody response.

As previously discussed, stress can suppress antibody and physical activity can have beneficial effects on survivability. The interaction of these conditions has thus far received little scientific attention. Research by Dishman and colleagues (9, 10) reported that
voluntary physical activity (activity wheel or treadmill running) for 6 wk before foot-shock stress attenuated the stress-induced suppression of natural killer cell cytotoxicity in rats. They concluded that voluntary physical activity could buffer the stress-induced suppression of immune function.

The effect of physical activity may have on stress-induced suppression of an in vivo measure of immunity, such as the antibody response, remains unknown. Several studies have addressed the impact of physical activity, per se, on the specific antibody response to antigen. However, the antibody response measured in these studies was often only measured at a single time point and only measured in healthy, uncompromised animals (1, 7, 22). What remains unclear, therefore, is whether prior physical activity can ameliorate the stress-induced suppression of a specific antibody response. To address this issue, we investigated the potential of prior voluntary freewheel running to prevent stress-induced suppression of the in vivo antibody response to KLH. We also examined whether prior freewheel running would also prevent the behavioral depression previously demonstrated to be produced by stress (26).

MATERIAL AND METHODS

Animals

Adult male Sprague-Dawley rats (300 g, Harlan Sprague Dawley, Indianapolis, IN) were used in all experimental procedures. All subjects were maintained on a 12:12-h light-dark cycle (lights on 0600–1800) and were allowed to acclimate to the colony for 2 wk before onset of any experimental manipulation. Standard laboratory rat chow (LabChow) and water were freely available. All animals were individually housed in hanging metal cages (25 × 15 × 13 cm, length × width × height) with attached running wheels (37.5-cm diameter) and allowed to habituate to the running wheel environment by being housed for 2 wk with a locked wheel. Wheels were rendered immobile with metal wire for control (sedentary) groups throughout the duration of the study. Colony room temperature was maintained at 22°C. Care and use of the animals were in accordance with protocols approved by the University of Colorado Institutional Animal Care and Use Committee.

Procedures

KLH immunization. All rats were immunized intraperitoneally with 200 μg soluble KLH (CalbioChem, lot No. 001738 in 50% glycerol) in 0.5 ml sterile saline. After immunization, rats were either returned to their cage or exposed to inescapable tail shock.

Stress protocol. Animals either remained in their cage (No stress) or were subjected to a tail-shock stress procedure lasting ~110 min. The stress procedure consisted of being lightly restrained in Plexiglas tubes (23.4 cm long and 7 cm in diameter) and exposed to 100 inescapable shocks (IS) of 5 s and 1.6 mA with an average intertrial interval of 60 s through electrodes attached to their tail. All animals were stressed during the light photoperiod between 0900 and 1200. After stressor termination, rats were returned to their cages. This stress procedure did not result in tissue damage to the animal.

Running activity. The impact of freewheel running on behavioral depression was tested by comparing the impact of stress in animals with prior wheel-running experience to those without. Rats (n = 8–10/group) had continuous access to a mobile running wheel 4 wk before IS stress. Running behavior was recorded daily for 8 wk. For comparison, the impact of stress on spontaneous wheel-running behavior was also tested in rats that had no prior wheel-running experience. Rats (n = 9–10/group) were either exposed to IS or remained in their home cage. Immediately after IS exposure, rats returned to their home cages and all of the running wheels were unlocked. Running behavior of the stressed (IS) and control rats was recorded daily for 4 wk.

The impact of freewheel running on stress-induced suppression of anti-KLH Ig was tested using a 2 (stress or home cage control) × 2 (sedentary or active) experimental design (n = 8–10/group). Only prior freewheel running was tested for anti-KLH Ig, because this procedure was effective at reducing behavioral depression caused by IS (see Fig. 1A).

Body weight. Animals were removed from their cages at weekly intervals, and body weight was determined to the nearest gram on an Ohaus triple-beam balance.

Blood sampling. A blood sample for antibody assessment was quickly taken (within 2 min of touching cage) by gently wrapping the rat in a small towel and lightly restraining it using a velcro strapping apparatus. A small nick was made in the exposed tail with a #15 scalpel, and a blood sample (300 μl) was taken and allowed to clot. The clot was discarded, and the serum was collected and frozen. Serum concentrations of anti-KLH Ig were measured using an in vivo antibody response to KLH.

Fig. 1. Average daily activity on voluntary running freewheels over a 7-day period is presented for each week of the study. Animals received inescapable tail-shock stress (IS) at the onset of voluntary activity (A) or were allowed 4 wk of voluntary activity before IS or shock (B). Arrow indicates day of IS administration.
anti-rat IgG 2a) with time as the repeated measure. Statistical significance was accepted at α = 0.05. Statistical analysis was conducted at 0900 on days 7, 14, 21, and 28 post-KLH immunization.

**Anti-KLH IgM assessment.** An ELISA was used for antibody assessment. Microtiter plates (96 well, Immulon-4, Dynex) were coated with 0.5-mg/ml dialyzed KLH for 3 days at 4°C. Plates were then washed and blocked with 5% BSA (Sigma) overnight at 4°C. Serum samples were diluted (IgM 1:400) in PBS containing 0.05% Tween 20 (Sigma). A single serial dilution (1:2) of these concentrations was performed. These dilutions ensured the sample concentration fell within the linear range of the plate reader. Microtiter plates were incubated for 3 h at 37°C and then washed with PBS-Tween mixture. Secondary antibody, alkaline phosphatase-conjugated goat anti-rat IgM (1:5,500 dilution, Cappel) was added to each well for 60 min at 37°C. Plates were again washed three times before addition of p-nitrophenyl phosphate substrate (Sigma). Plates were incubated at room temperature in the dark until plate-positive control wells registered an optical density of ~1.0 at 405 nm on a Dynatech plate reader. Plate-positive control wells consisted of sera pooled from rats (15–20 KLH-immunized Sprague Dawley). This served as an internal control to minimize plate-to-plate variability inherent to the assay. Results are presented as a decimal of this positive standard (Sample OD/Positive OD).

**Anti-KLH IgG1 and IgG2a assessment.** KLH-coated microtiter plates were prepared and blocked with BSA as described above. Serum samples were diluted 1:3,000 with PBS-Tween mixture before addition to wells. Plates were incubated at 37°C for 3 h before washing three times with PBS-Tween mixture. Secondary antibody consisted of either horseradish peroxidase-conjugated mouse anti-rat IgG1 (1:1,000, Zymed) or horseradish peroxidase-conjugated mouse anti-rat IgG2a (1:2,000, Zymed). Incubation with the secondary antibody was for 60 min at 37°C. The plate was then washed three times before addition of [2,2’azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] substrate (Zymed). Incubation of substrate continued until plate-positive control wells registered an optical density of 1.0 on a 405-nm filter (Dynatech plate reader). Plate-positive control wells consisted of sera pooled from rats (15–20 KLH-immunized Sprague Dawley). This served as an internal control to minimize plate-to-plate variability inherent to the assay. Results are presented as a decimal of this positive standard (Sample OD/Positive OD).

**Statistical Analysis**

Repeated-measure ANOVAs were performed for all analyses (body wt, running distance, anti-KLH IgM, IgG1, and IgG2a) with time as the repeated measure. Statistical significance was accepted at α = 0.05. SuperAnova for Macintosh computers was used for the analyses. Post hoc analyses used the Fisher’s protected least significant difference (F-PLSD) test.

**RESULTS**

**Voluntary Running**

Weekly running distances are presented in Fig. 1. Voluntary wheel-running behavior is reduced by IS for 4 wk after IS exposure in rats without prior wheel-running experience (Fig. 1A). This is supported by a statistically significant main effect of stress \( F(1,18) = 4.4, P < 0.05 \). Figure 1B shows the protective effect of prior freewheel running on stress-induced behavioral depression. Rats allowed to run for 4 wk before stress recover more quickly than rats not allowed to run before stress (Fig. 1B). The stress-induced behavioral depression is recovered 1–2 wk after stress (day 42) in rats with prior wheel-running experience. This is supported by a statistically significant stress × time interaction \( F(7,112) = 2.4, P < 0.05 \). Post hoc analyses (F-PLSD) revealed a reliable reduction in running 1 wk (day 35) after IS (\( P < 0.05 \)) but not 2 (day 42), 3 (day 49), or 4 (day 56) wk after IS (\( P > 0.05 \)). In both studies, average weekly running increased across the first 4 wk [Fig. 1A: \( F(3,54) = 7.3, P < 0.001 \); Fig. 1B: \( F(7,112) = 7.6, P < 0.001 \)]. In both groups, running activity for nonstressed rats, as measured by kilometers run per week, increased over the first 3 wk with a peak average distance of 14–15 km/wk. If running continued beyond 4 wk, average weekly distance run decreased over the next 5 wk, ending with 9.7 km/wk in week 8 (Fig. 1B). Decreases in voluntary running activity after ~4 wk of activity are frequently noted in rats fed ad libitum, as was done in the present study (31).

**Body Weight**

Figure 2 presents change in body weight from the start of the study. Both tail-shock stress and freewheel running reduce body weight gain, and the effect of both stress and physical activity changed across time. This is supported by statistically significant stress × time \( F(8,288) = 3.0, P < 0.01 \) and activity × time \( F(8,288) = 4.1, P < 0.001 \) interactions. The effect of stress on body weight loss was not affected by prior freewheel running. There were no statistically significant stress × activity interactions.

**Anti-KLH Immunoglobulin (IgM, IgG1, and IgG2a)**

As seen in Fig. 3, stress suppressed the anti-KLH IgM response in sedentary rats (Fig. 3A) in agreement

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**Figure 2.** Rat body wt was measured at weekly intervals to the nearest gram. Data are presented as weight change in grams from baseline (BL) body wt. Four weeks after activity onset (Active) or no activity (Sedentary), IS or no stress (HCC) was administered.
with published literature (17, 18). However, rats that were physically active for 4 wk before stressor exposure had no stress-induced suppression in anti-KLH IgM (Fig. 3B). This was supported by a statistically significant stress × activity interaction \([F(1,32) = 9.0, P < 0.01]\). There were no statistically significant effects of exercise alone. These data have been presented in part elsewhere (16).

We next investigated the effect of stress and physical activity on anti-KLH IgG1 (Fig. 4) and IgG2a (Fig. 5). As shown in Fig. 4, anti-KLH IgG1 is not suppressed by stress \([F(3,96) = 1.26, P = 0.29]\). This finding was expected because published research from our lab has suggested that the IgG1 isotype is stress insensitive (17, 18). Interestingly, although physical activity alone did not influence the IgG1 response, physical activity in conjunction with stress resulted in an elevation of anti-KLH IgG1 that increased across time. This was supported by a statistically significant activity × time interaction \([F(3,96) = 3.75, P < 0.01]\).

Anti-KLH IgG2a data are presented in Fig. 5. Stress suppressed the anti-KLH IgG2a response in sedentary rats (Fig. 5A), in agreement with published literature (17, 18). However, rats who were physically active for 4 wk before stressor exposure had no stress-induced suppression in anti-KLH IgG2a (Fig. 5B). This was supported by a statistically significant stress × activity × time interaction \([F(3,96) = 3.3, P < 0.05]\). Physical activity alone had no effect on anti-KLH IgG2a (\(P > 0.05\)).

**DISCUSSION**

Results from this study, as well as previous findings from our laboratory (16–18, 26), support the conclusion that exposure to stress (IS) leads to a long-term (4 wk) reduction in spontaneous wheel-running behavior (Fig. 1B) and anti-KLH Ig (Figs. 3A and 5A). Voluntary freewheel running before exposure to stress (IS) prevented both the stress-induced reduction in wheel-running behavior and the suppression of the anti-KLH antibody response. These data suggest that physical activity before stress blunts the behavioral depression and immunosuppression produced by stress. The mechanism(s) responsible for this effect are unknown.

There are, however, several potential immunological mechanisms for the stress-buffering effect of freewheel running. The KLH antibody response requires the interaction of antigen-presenting cells, T helper cells (\(T_{h1}\) and \(T_{h2}\)) and B cells. Fleshner et al. (18) reported that stress (IS) reduced the KLH-stimulated increase in \(T_{h1}\) splenocyte numbers and interferon gamma (IFN-\(\gamma\))
production. IFN-γ is a Th1 cell cytokine important for the antibody class switch from IgM to IgG2a (18), and IS results in a selective suppression in anti-KLH IgG2a. We have proposed that stress reduces anti-KLH IgG2a because Th1 cells are particularly stress sensitive (18). Thus stress at the time of KLH immunization leads to a reduction in anti-KLH Th1 cells, less IFN-γ, and less anti-KLH IgG2a. Given that physical activity prevented the stress-induced suppression in anti-KLH IgG2a and had little effect on anti-KLH IgG1, it is possible that physical activity prevents the stress-induced reduction in anti-KLH Th1 cells. We are currently investigating this idea.

In addition to direct immunological mechanisms for the stress-buffering effect of physical activity, neuroendocrine changes in physically active rats could also play a role. Although adaptations to the hypothalamus-pituitary-adrenal (HPA) axis have been reported with some types of physical activity (38), the stress-buffering effect of freewheel running is likely not due to a reduction in HPA response to stress. This is true because both physically active and sedentary rats have similar corticosterone responses to IS. Adult male rats (n = 8/group) were allowed to run for 4 wk or remained sedentary. Tail vein samples were taken before stress [baseline (BL)], after 100 tail shocks (IS) of 1.6 mA, or 24 h after the termination of tail shock (Post). The corticosterone levels for sedentary rats were the following: BL = 5.9 ± 1.4 µg/dl, IS = 63 ± 3 µg/dl, and Post = 13 ± 2 µg/dl. The corticosterone response for the active rats was the following: BL = 8 ± 1 µg/dl, IS = 59 ± 2 µg/dl, and Post = 13 ± 2 µg/dl.

Although changes in the glucocorticoid response to stress may not be a mechanism for the stress-buffering effect of physical activity, catecholamines, specifically norepinephrine (NE), released from sympathetic nerve terminals in immune organs may be relevant. Lymphoid organs, including the lymph nodes and spleen, are highly innervated (13, 14). These sympathetic nerve terminals are also situated in close approximation to T helper cells that reside in the germinal center (13, 14). In addition, both α- and β-adrenergic receptors have been measured on mouse B and Th1 but not Th2 cells (23, 32). The source of the immunomodulating catecholamine is the splenic nerve, because severing the splenic nerve before foot-shock stress and sheep red blood cell (SRBC) antigen administration eliminates the stress-induced suppression in anti-SRBC IgM-producing cells (37). This finding suggests that splenectomy may be important in the immune response where the Th1 cell has a pivotal role, such as in the study presented here. Finally, we have preliminary evidence to suggest that prior freewheel running may, in fact, reduce splenic NE output during IS (16), and this reduction could be an important neuroendocrine mechanism whereby freewheel running prevents stress-induced suppression of anti-KLH Ig.

Although the distances run by the rats in this study are relatively low, they are within the range expected of adult male Sprague-Dawley rats (20, 21, 30, 33). In addition, health-related benefits have been previously reported for low-activity levels. For example, a study by Cohen et al. (6) found reductions in tumor cell foci in rats with a voluntary daily activity of 1.6 km; a second study found improvements in systolic blood pressure and serum lipids in spontaneously hypertensive rats and serum lipids in spontaneously hypertensive rats that voluntarily ran only 722 m/day (6, 36). In addition, endocrine and oxidative enzyme adaptations in Sprague-Dawley rats, with voluntary running activity similar to levels found in this study (2.1 km/day), have been reported (29, 33). Therefore, low-activity running programs appear to have a sufficient stimulus to promote physiological adaptations resulting in improved health parameters.

The study presented here suggests that moderate physical activity can prevent stress-induced behavioral depression and immunosuppression. Specifically, prior freewheel running reduced the behavioral depression produced by stress from 4+ wk to 1–2 wk and prevented the stress-induced suppression of KLH-specific IgM and IgG2a. The potential neuro-endocrine-immune mechanisms for this effect are currently under investigation.
REFERENCES


