Role of extracellular HSP72 in acute stress-induced potentiation of innate immunity in active rats

J. CAMPISI AND M. FLESHNER
Department of Kinesiology and Applied Physiology, and Center for Neuroscience, University of Colorado at Boulder, Boulder, Colorado 80309-0354
Submitted 25 July 2002; accepted in final form 9 September 2002

Campisi, J., and M. Fleshner. Role of extracellular HSP72 in acute stress-induced potentiation of innate immunity in active rats. J Appl Physiol 94: 43–52, 2003. First published September 13, 2002; 10.1152/japplphysiol.00681.2002.—Acute stress can compromise acquired, and potentiate innate, immunity. Recent evidence suggests that the impact of stress on measures of immunity can be modulated by the physical activity status of the organism and that extracellular heat shock protein 72 (eHSP72) contributes to the activation of innate immunity produced by stress. Therefore, this study investigated whether physical activity status would impact the immunologically enhancing effects of stressor exposure (inescapable tail-shock stress [IS]) on innate immunity and whether changes in eHSP72 responses could play a role. Adult, male Fischer 344 rats lived with mobile (physically active) or immobile (sedentary) running wheels. After 6 wk, rats were exposed to IS or to no stress. Immediately after IS, all rats were injected subcutaneously with live Escherichia coli. Inflammation was assessed daily, and plasma eHSP72 was measured at various time points. Rats exposed to IS resolved their inflammation faster than nonstressed rats, but the beneficial impact of stress on recovery was greater in physically active rats. All rats had equal increases in circulating eHSP72 after IS. Splenocytes harvested from a separate cohort of nonstressed rats were cultured with eHSP72, and nitric oxide and cytokines were measured. Physically active rats responded to eHSP72 stimulation in vitro with a greater nitric oxide and cytokine response than sedentary rats. Thus physically active rats both recover faster than sedentary rats after bacterial challenge + IS exposure and demonstrate potentiated cellular responses to eHSP72 activation that could be important for bacterial recovery.

heat shock proteins; inflammation; Escherichia coli; physical activity

IT IS NOW WELL ESTABLISHED that stress can alter immune function (1, 38). The impact of stress on measures of immunity is affected by many variables, including characteristics of the stressor (duration, intensity), the immune outcome measure (innate, acquired), and attributes of the organism (age, physical activity status) (15, 38). Accordingly, alteration of any of these variables profoundly influences the effect of stress on the immune system. For example, moderate voluntary physical activity can prevent many of the immunologically suppressive consequences of stress on acquired immunity (5, 10, 23, 24, 26, 40).

Although the impact of acute stress on immunity is complex, the majority of research regarding stress and immunity has focused on the compromising effects of stress on acquired immunity (1). Recently, it is becoming clear that, unlike acquired immunity, innate immunity may be enhanced after exposure to an acute stressor. For example, exposure to acute laboratory stressors can increase fever (18), prostaglandin E2 release (33, 41), macrophage/neutrophil nitric oxide (NO) production (17, 30) and phagocytosis (32, 37), proinflammatory cytokine release (34, 43, 47, 52), acute phase proteins (18, 29), complement activity (16, 28), and the mobilization of leukocytes from the blood to the site of challenge (20). In addition, recent studies have revealed that exposure to acute laboratory stressors can alter host resistance to bacterial infection. For example, Campisi et al. (12) recently reported that exposure to 100 min of intermittent inescapable tail-shock stress (IS) reduced inflammatory size, the time required for complete inflammation resolution, and body weight loss after subcutaneous injection of live (i.e., replicating) bacteria (Escherichia coli). Similar observations were reported by Deak et al. (19) after IS and administration of streptomycin-treated (i.e., nonreplicating) E. coli. The inflammatory response is an excellent measure of innate immune system effectiveness because E. coli-induced inflammation is generated and resolved primarily by neutrophils and macrophages, the two primary cells of innate immunity (2). When the complexity of the immune system and the diverse components of innate immunity that are elevated by acute stress are taken into consideration, it is possible, and even probable, that numerous mechanisms contribute to stress-induced enhancement of innate immunity.

One mechanism that has recently received attention is the role of extracellular heat shock proteins (eHSP) in stress-induced immunomodulation (12, 13). eHSP are released after acute stress and can, in the absence

Address for reprint requests and other correspondence: M. Fleshner, Dept. of KAPH/Center for Neuroscience, Campus Box 354, Univ. of Colorado at Boulder, Boulder, CO 80309-0354 (E-mail: Fleshner@spot.colorado.edu).

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of a foreign pathogen, modulate the innate immune system. Specifically, under pathophysiological conditions of cellular stress, eHSP are released from cells into the extracellular environment (7, 13) and promote local inflammation through activation of the innate immune system (36). We (13) and others (4, 7, 14, 36, 44) have reported that eHSP can activate innate immune cells, which results in the production of proinflammatory cytokines and NO in vitro. Furthermore, recent evidence indicates that eHSP can also increase the rate of phagocytosis (35) and activate the complement system (45, 46). These observations have led to the hypothesis that stress-induced release of eHSP potentiates components of innate immunity and contributes to the improved bacterial killing and facilitated recovery after bacteria challenge (Fig. 1) (13). In fact, there are data to support this hypothesis. We have recently reported that eHSP72 administration in vivo facilitates recovery from a bacterial challenge and stimulates the release of bactericidal NO production. We have proposed that the facilitation in NO contributes to the beneficial effects of stress because inhibition of the stress-induced enhancement of NO attenuates the facilitated pattern of recovery (13).

Interestingly, the impact of stress on measures of innate immunity can be changed by the physical activity status of the organism. For example, it has recently been reported that prior free wheel running facilitates the stress-induced release of neutrophils into the circulation and into the inflammatory site (28). However, it remains unknown what role, if any, eHSP may play in modulating these effects. The present set of studies, therefore, examined the effect of acute stress on the inflammatory response after subcutaneous challenge with replicating bacteria (*E. coli*) and the effect of eHSP72 on NO and cytokine responses in both physically active (PA) and sedentary (Sed) rats. We hypothesize that habitual, moderate, voluntary physical activity will improve both the beneficial effect of stress on bacterial infection recovery and the eHSP72-induced release of NO and proinflammatory cytokines.

**METHODS**

**Animals.** Adult (3 mo old at start of experimentation) male viral-free Fischer 344 rats (203 ± 2 g; Harlan Labs) were individually caged in Plexiglas cages (60 × 30 × 24 cm) with food and water available ad libitum. Animal colonies were maintained in a pathogen-free barrier facility with a 12:12-h light-dark cycle (lights on 0600–01800). Rats were given at least 1 wk to habituate to the colonies before experimentation. Body weights were monitored daily. All rats were handled and weighed each day for at least 3 days before each study began (5 rats per experimental group). Care and use of the animals were in accordance with protocols approved by the University of Colorado Institutional Animal Care and Use Committee.

**IS procedure.** Animals either remained undisturbed in their home cages as controls (HCC) or were exposed to IS as described previously (12, 30). IS rats were transported to an adjacent room and placed in Plexiglas restraining tubes (15 × 7 cm). Contact beams were placed on the tail of the rat, and 100 1.6-mA tailshocks (5-s duration, variable intertrial interval of 60 s; range 30–90 s) were administered. The total IS session lasted 100 min. All animals were shocked between 0800 and 1000.

**Free wheel running.** Rats were individually caged in Nalgene Plexiglas cages (45 × 25.2 × 14.697 cm) with a stainless steel open running wheel attached (1.081-m circumference). PA rats had a mobile running wheel and ran for 6 wk before stressor exposure. Sed rats were housed in the same environment except that the running wheel was locked and remained immobile. Voluntary free wheel running was the chosen modality because, in contrast to forced treadmill training, voluntary free wheel running does not produce negative adaptations (i.e., adrenal hypertrophy, thymic involution, decreased corticosteroid binding globulin, elevated lymphocyte nitrite concentrations, and suppressed lymphocyte proliferation) that are indicative of chronic stress in rats (39, 40). Furthermore, this specific modality of physical activity is sufficient to modulate many physiological and immunological responses after stressor exposure (22, 27, 28, 40). The caging environment meets National Institutes of Health floor space standards for a single rodent. Total daily running distances were monitored by computer with the VitalView Automated Data Acquisition System (Sunny River, OR).

![Fig. 1. Schematic illustration of how stress-induced extracellular heat shock protein (eHSP) 72 could be facilitating host defense.](image-url)

Acute stress increases eHSP72 that accumulates at the site of inflammation and potentiates bacterial-stimulated nitric oxide (NO) and proinflammatory cytokine release by macrophages and/or neutrophils. Potentiated NO and proinflammatory cytokines improve bacterial killing and lead to faster resolution of the bacteria, restricted inflammation, and overall improved host defense. TNF-α, tumor necrosis factor-α; IL, interleukin.
Plasma collection and eHSP72 measurement. Plasma was collected from rats the day before exposure to IS (baseline), after 25 shocks (25 min after initiation of IS), after 100 shocks (100 min after initiation of IS), and 2 and 48 h after the termination of IS. The rat was removed from its home cage, gently wrapped in a towel, and lightly restrained with a Velcro strap. The tail was exposed, a small nick was made in a lateral tail vein with a scalpel (no. 15 blade), and the tail was gently stroked until a volume of ~300–300 μl of whole blood was obtained in EDTA (Sigma Chemical)-treated microfuge tubes. Samples collected from rats during IS required that the rats remain in the IS restraint tubes and the contact beams be temporarily removed while the tail nick was performed. The entire sampling procedure was accomplished within 2 min of approaching the cage/restraint tube. Samples were spun in a refrigerated centrifuge immediately, and plasma was aliquoted and stored at −20°C until the time of assay. Heat shock protein (HSP) 72 was measured from plasma by using a commercially available ELISA for rat eHSP72 (StressGen Biotechnologies). Samples were assayed at optimal concentrations and according to the manufacturer’s instructions.

Bacterial culture. E. coli (ATCC 15746) was purchased from American Type Culture Collection (Bethesda, MD). Vial contents were rehydrated and grown overnight to maximal densities in 30 ml of brain-heart infusion (DIFCO Laboratories, Detroit, MI) at 37°C, 95% air + 5% CO2. Cultures were then aliquoted into 1.0 ml of brain-heart infusion supplemented with 10% glycerol and frozen at −70°C. These vials constituted the stock cultures. All experiments utilized bacteria from these stock cultures. One day before experimentation, stock cultures were thawed and cultured overnight (~19 h) in 35 ml of brain-heart infusion (37°C, 95% air + 5% CO2). The number of bacteria in cultures was quantified by extrapolating from previously determined growth curves, as previously described (12). Cultures were then centrifuged for 15 min at 3,000 rpm, supernatants were discarded, and bacteria were resuspended in sterile PBS. All rats were injected with ~2.5 × 109 colony-forming units of E. coli because previous work in our laboratory determined that this dose produces a reliable inflammatory response (12). Although gram-positive bacteria such as Staphylococcus aureus are more likely to infect the skin than gram-negative bacteria such as E. coli, previous work (19) indicated that a subcutaneous injection of E. coli produced a more consistent and robust inflammatory response than did S. aureus. Furthermore, whenever one administers gram-positive bacteria to the skin of a rodent, issues of contamination arise because opportunistic gram-positive bacteria infect the area, making interpretation of the quantity/killing/clearance of the administered bacteria difficult (49).

Bacterial challenge and in vivo inflammation assessment. One day before injection, an area on the rat’s dorsal surface that measured ~5.0 × 7.0 cm was shaved so that inflammation could easily be quantified. On the day of experimentation, injections were given subcutaneously in a volume of 250 μl in the center of the back immediately after IS or HCC treatment. The diameter of inflammation (to the nearest tenth of a millimeter) was measured daily between 1000 and 1100 h using a digital caliper. Bacterial inflammation grade measurements were also taken to indicate the severity of inflammation. The grade of inflammation was determined daily on a scale of 0–4 with 0 = normal, 1 = light pink, 2 = pink, 3 = red, and 4 = dark red/purple. All measurements were made on the same relative region of the dorsum by an independent observer who was blind to the experimental condition of the rat.

RESULTS

Free wheel running. Weekly running distances are presented in Fig. 2. Fischer rats in the present studies voluntarily ran in their wheels an average of 17.9 ± 1.3 km/wk. Running activity, as measured by kilometers run per week, increased over the first 6 wk and averaged 17.6 km/wk. Bacterial challenge resulted in significant reductions in wheel running, and the sickness-induced, decreased voluntary running distance was recovered 1 wk after challenge. This is supported by a statistically significant main effect of time (P1,18 = 7.668, P = 0.02). Post hoc analyses (Fisher’s paired least-significant difference) revealed a reliable reduction in running the week after bacterial challenge and stress (week 7) for both HCC and IS rats (P > 0.05). However, no differences existed between rats exposed to stress and challenged with live bacteria and rats exposed to no stress and challenged with bacteria. One week after bacteria challenge and IS or HCC, rats resumed their previous pattern of voluntary activity,
which averaged 18.2 km/wk for the duration of the study with no differences between the groups.

**Body weight.** Figure 3 presents body weight data. All rats increased their body weight throughout the course of the study. Bacterial challenge alone and bacterial challenge plus tail-shock stress resulted in a reduction in body weight. This is supported by a statistically reliable main effect of time \( F(34,544) = 1.441, P = 0.05 \) and grade \( F(34,544) = 1.476, P = 0.04 \). Fisher's paired least-significant difference test revealed a reliable difference between the impact of stress on inflammation diameter in the PA rats vs. Sed rats on day 5 \( P = 0.01 \), day 6 \( P = 0.005 \), day 8 \( P = 0.03 \), and day 9 \( P = 0.03 \) after stress and *E. coli* challenge. Fisher's paired least-significant difference test revealed a trend toward a

(day 34; PA mean = 299.1 ± 7.4 g; Sed mean = 294.9 ± 7.1 g).

**Plasma eHSP72 measurement.** A significant main effect of group [IS vs. HCC; \( F(1,12) = 41.64; P < 0.0001 \)] and group \( \times \) time interaction \( F(4,48) = 12.024; P < 0.001 \) indicated that IS resulted in an increase in eHSP72. Post hoc analysis revealed that levels of eHSP72 increased rapidly after the onset of stress (25 min; \( P = 0.003 \)), remained elevated for at least 2 h after stressor termination (\( P = 0.0006 \)), and returned to baseline values 48 h after stressor termination (Fig. 4). Importantly, both PA and Sed rats demonstrated equal amounts of circulating eHSP72 after IS at all time points.

**Bacterial challenge and in vivo inflammation assessment.** Inflammation diameter (Fig. 5, A and B) and grade (Fig. 5C) were greatly increased 1 day after subcutaneous injection of live *E. coli*. Stress before *E. coli* reliably reduced the size [IS vs. HCC; \( F(1,16) = 48.139; P < 0.0001 \)] and grade [IS vs. HCC; \( F(1,16) = 8.963; P = 0.0086 \)] of the inflammatory response. Physical activity alone had no effect on inflammation. The effect of stress in the PA rats was increased such that PA and stressed rats had the greatest reduction in bacterial inflammation diameter [stress \( \times \) activity: \( F(1,16) = 6.216, P = 0.02 \); stress \( \times \) activity \( \times \) time: \( F(34,544) = 1.441, P = 0.05 \)] and grade [activity \( \times \) time: \( F(34,544) = 1.476, P = 0.04 \)]. Fisher's paired least-significant difference test revealed a reliable difference between IS and HCC animals. #Significant difference between IS and HCC [Fisher's protected least-significant difference (F-PLSD) test; \( P < 0.05 \)]. HSP, heat shock protein. Values are means ± SE.
reliable difference between the impact of stress on inflammation grade in the PA vs. Sed rats on day 1 ($P = 0.07$) after stress and *E. coli* challenge. Analyses of the time required for complete inflammation resolution (defined as the total number of days needed to achieve basal cutaneous thickness) indicated that rats exposed to stress resolve their inflammation faster than nonstressed rats ($F(3,16) = 7.26, P = 0.002$), and PA rats exposed to stress completely resolve inflammation faster than Sed stressed rats ($P = 0.03$) (Fig. 5C).

**NO and cytokines.** We (13) and others (3, 4, 8, 42) have recently demonstrated that recombinant eHSP72 (StressGen Biotechnologies) stimulates NO and proinflammatory cytokine production from leukocytes. The results from the present studies confirm and extend these findings. Splenocytes responded to stimulation with eHSP72 in culture by producing nitrite and proinflammatory cytokines (Fig. 6). Analysis revealed a significant main effect of treatment (media vs. eHSP72) for nitrite ($F(1,16) = 26.486, P \leq 0.0001$), TNF-$\alpha$ [$F(1,16) = 60.618, P \leq 0.0001$], IL-1$\beta$ [$F(1,16) = 18.68, P = 0.0005$], and IL-6 [$F(1,16) = 82.825, P \leq 0.0001$]. The effect of eHSP72 stimulation in the PA rats was increased such that PA rats had greater induction of nitrite [PA vs. Sed: $F(1,16) = 6.945, P = 0.01$], IL-1$\beta$ [PA vs. Sed: $F(1,16) = 9.688, P = 0.0067$], and IL-6 [PA vs. Sed: $F(1,16) = 6.661, P = 0.02$] and a trend for greater TNF-$\alpha$ [PA vs. Sed: $F(1,16) = 3.584, P = 0.07$].

Similarly, splenocytes responded to stimulation with LPS in culture by producing nitrite and proinflammatory cytokines (Fig. 7). A significant main effect of treatment (media vs. LPS) for nitrite [$F(1,16) = 193.745, P \leq 0.0001$], TNF-$\alpha$ [$F(1,16) = 115.547, P \leq 0.0001$], IL-1$\beta$ [$F(1,16) = 39.127, P \leq 0.0001$], and IL-6 [$F(1,16) = 566.637, P \leq 0.0001$] was found for spleen cells. Although the effect of eHSP72 in the PA rats was increased such that PA rats demonstrated potentiated responses, this was not the case for LPS. Both PA and Sed rats made equal amounts of nitrite and cytokines after stimulation with LPS alone. However, when spleen cells were stimulated with LPS combined with eHSP72, PA rats once again demonstrated potentiated responses (Fig. 8, A–D). Analysis revealed a significant main effect of treatment (media vs. LPS + eHSP72) for nitrite [$F(1,16) = 57.939, P \leq 0.0001$], TNF-$\alpha$ [$F(1,16) = 63.286, P \leq 0.0001$], IL-1$\beta$ [$F(1,16) = 25.341, P \leq 0.0001$], and IL-6 [$F(1,16) = 316.55, P \leq 0.0001$]. The effect of LPS + eHSP72 stimulation in PA rats was increased such that PA rats had greater induction of nitrite [PA vs. Sed: $F(1,16) = 8.104, P = 0.01$] and IL-6 [PA vs. Sed: $F(1,16) = 7.801, P = 0.01$] and a trend for greater TNF-$\alpha$ [PA vs. Sed: $F(1,16) = 115.547, P \leq 0.001$] and grade (0 to 4 rating; $P = 0.02$) and a trend for greater TNF-$\alpha$ [PA vs. Sed; $F(1,16) = 115.547, P \leq 0.001$] and grade (0 to 4 rating; $P = 0.02$). *Significant difference between IS and media (F-PLSD; $P < 0.05$)."
studies was tested for endotoxin by using a limulus amebocyte lysate assay and tested the ability of HSP72 to stimulate cells in the presence of polymyxin B, a potent LPS inhibitor (25, 31). HSP72 contained very little endotoxin (<1.0 EU/10 µg), and stimulation of cells in the presence of polymyxin B abrogated LPS-induced but not HSP72-induced cytokine production (13). Furthermore, boiling HSP72 at 100°C for 90 min before stimulation completely prevented cytokine production (13). Thus we are confident that the effect of eHSP72 on nitrite and proinflammatory cytokines is not because of endotoxin contamination and is a specific effect of the protein.

**DISCUSSION**

Many variables impact the effectiveness of the immune system in host defense. In the present series of studies, we demonstrate that both the stress status and physical fitness level of an organism have pronounced effects on the function of the immune system. As shown in Fig. 5 and consistent with previous work (12), acute stressor exposure (IS) facilitates innate immunity such that Sed, stressed rats completely resolve inflammation after a live bacteria challenge faster than their nonstressed Sed controls (12% faster, 4 days faster). PA rats that were exposed to stress resolved inflammation even faster (21% faster, 7 days faster). Although both Sed and PA stressed rats had reductions in inflammation diameter, PA stressed rats demonstrated a steeper slope of recovery, which resulted in shorter time to recovery. These data support the conclusion that the beneficial impact of stress on bacterial recovery was greater in PA than in Sed stressed rats. A similar observation has been reported after challenge with nonreplicating bacteria (28), which suggests the generalizability of these results, but it is currently unknown whether these effects occur when other strains of bacteria are used to induce inflammation. Studies are presently underway to address this question. These data support the hypothesis that the positive effects of stress on innate immunity are facilitated in PA organisms.

**Fig. 6.** Spleen cells (5 × 10⁶ cells/ml) were harvested from nonstressed rats that were allowed to live with either a mobile (PA) or locked (Sed) running wheel for 6 wk. Cells were stimulated in the presence of recombinant eHSP72 (10 µg/ml) or media control. After 24 h of incubation, cells were harvested, and nitrite (A) and proinflammatory cytokines (TNF-α (B), IL-1β (C), IL-6 (D)) were measured. Values are means ± SE. *Significant difference between eHSP72 and media stimulation (F-PLSD; P < 0.05). #Significant difference between PA and SED rats (F-PLSD; P < 0.05).
results found. The focus of this study, however, was on the effect of physical activity on the eHSP72 response. We chose to focus on this response because we have recently reported that eHSP72 contributes to the stress-induced facilitation in recovery from *E. coli* infection in Sed animals (13), and a comparison of the eHSP72 response in PA vs. Sed animals has not been previously reported.

Recent evidence indicates that innate immune cells express surface receptors that bind eHSP. Asea et al. (4) reported that eHSP70 specifically binds to the surface of monocytes and elicits a proinflammatory cytokine response through a cluster differential (CD) 14-dependent pathway and subsequent activation of NF-κB. Furthermore, CD91 (α2 macroglobulin receptor) has been reported to be a receptor for eHSP on macrophages (6). In light of the facts that eHSP bind and activate innate immune cells and PA stressed rats demonstrate facilitated innate immune responses, we examined whether PA rats released more eHSP after stress. Interestingly, PA and Sed rats released equal amounts of eHSP72 in the circulation after exposure to acute stress (Fig. 4). Thus any change in the inflammatory response cannot be attributed to an increase in eHSP72 release. Importantly, PA rats responded to eHSP72 stimulation with more cytokines and NO compared with Sed rats (Fig. 6). In addition, PA rats also responded with more cytokines and NO after LPS+eHSP72 stimulation (Fig. 8). It is possible, therefore, that one potential mechanism for facilitated recovery from bacterial infection in PA stressed rats is a greater NO-cytokine response to a given unit of eHSP72. Increased concentrations of NO or proinflammatory cytokines would likely facilitate bacterial killing and recovery. Numerous reports indicate that NO has potent antimicrobial properties (9) and that proinflammatory cytokines play both direct and indirect roles in combating bacterial infection (21). Indeed, inhibiting NO (13) and proinflammatory cytokine (11) production after bacterial challenge has been reported to slow recovery from infection.

eHSP stimulation of NO and proinflammatory cytokines could be playing a role in promoting recovery after bacteria challenge, but other mechanisms likely contribute to this effect. We have previously reported that exposure to IS in Sed organisms increases a wide variety of innate immune components, such as acute phase proteins (18), C3 function (28), and total circu-
lating white blood cells and neutrophils (28). Furthermore, exposure to inescapable shock has been reported to increase the rate of phagocytosis (32, 37). Increases in any of these components of innate immunity could feasibly contribute to the improvements in bacterial inflammatory responses observed after IS. Although not addressed in the present study, it is possible that eHSP72 might be responsible for many of the previously observed increases in components of innate immunity. In fact, there is evidence that eHSP can activate the complement system (45, 46) and increase phagocytosis (35) in vitro.

Physical activity, per se, had no effect on inflammation as PA rats that were not exposed to stress had no change in recovery from bacterial challenge. This is in agreement with previous work conducted with streptomycin-treated, nonreplicating bacteria (28). These data can be explained, in part, by examining the manner in which PA and Sed rats responded to eHSP72 and LPS stimulation. PA rats’ splenocytes responded to eHSP72 stimulation with a larger cytokine and NO response compared with those of Sed rats (Fig. 6). However, both PA and Sed rats’ splenocytes responded to LPS stimulation equally well (Fig. 7). Recall that HSP are only released from cells during times of stress (7). Therefore, it follows that, in the absence of stress, and thus in the absence of eHSP72 release, recovery from bacteria-induced inflammation would not be potentiated in a PA rat. This appears to be the case.

Bacterial challenge resulted in a small but significant reduction in wheel running in both stress and nonstressed rats. All rats, however, resumed the normal levels of activity 1 wk after bacterial challenge (Fig. 2). Bacterial challenge also resulted in significant reductions in body weight in all rats regardless of stress status (Fig. 3). Interestingly, despite the fact that they ran an average of 17.9 ± 1.3 km/wk, PA rats weighed the same as Sed rats throughout the study.

Clearly, numerous adaptations are incurred by physical activity that could contribute to an improved response after bacterial challenge and stress. In fact, it has been previously reported that PA stressed rats demonstrate greater neutrophils in the blood and at the site of benign E. coli challenge compared with their nonstressed counterparts (28). The initial stage of bacterially induced inflammation involves the migration of
of neutrophils/monocytes to the site of challenge (2) where, on stimulation via a variety of mechanisms (50), they release numerous bactericidal substances, such as NO, hypochlorous acid, and inflammatory regulatory proteins (48). Because PA rats appear better able to mobilize and migrate neutrophils to the site of challenge and PA rats’ cells respond to eHSP72 stimulation greater, it is likely that these two adaptations converge and result in improved bacteria lysis and clearance and, subsequently, contribute to faster inflammation resolution.

Although these studies demonstrate evidence supporting a theoretically likely mechanism for facilitated recovery after bacterial challenge in PA rats exposed to IS, they fail to show direct evidence linking eHSP72 to potentiated innate immunity in vivo. In light of the fact that recent evidence indicates the existence of a cell surface receptor for HSP70 on macrophages (51), which was later identified as the α2-2-macroglobulin receptor (6), future work will attempt to specifically block this receptor to examine its role in potentiating innate immune responses.

The present studies support the conclusion that acute stress facilitates features of innate immunity and that physical activity status modulates the impact of stress on immunity. Furthermore, these results indicate a novel adaptation at the cellular level to voluntary free wheel running. Rats that engage in habitual, voluntary, physical activity are more sensitive to eHSP72 stimulation. Increased cellular sensitivity, and subsequent greater magnitude of activation, would be adaptive to the organism if after stressor exposure they were exposed to a pathogenic challenge.

REFERENCES


