Voluntary wheel running initially increases adrenal sensitivity to adrenocorticotropic hormone, which is attenuated with long-term training

Jonathan E. Campbell,* Nasimeh Rakhshani,* Sergiu Fediuc, Silvio Bruni, and Michael C. Riddell

School of Kinesiology and Health Science, Muscle Research Centre, Faculty of Health, York University, Toronto, Ontario, Canada

Submitted 22 August 2008; accepted in final form 10 November 2008

Voluntary wheel running initially increases adrenal sensitivity to adrenocorticotropic hormone, which is attenuated with long-term training. *J. E. Campbell and N. Rakhshani contributed equally to this work. Address for reprint requests and other correspondence: M. C. Riddell, School of Kinesiology and Health Science, 4700 Keele St., Toronto, ON, Canada M3J 1P3 (e-mail: mriddell@yorku.ca).

The hypothalamic-pituitary-adrenal (HPA) axis is a vital component of the body’s stress response system. This pathway is activated by a variety of stressors that increase the pituitary release of adrenocorticotropic hormone (ACTH) into the circulation. ACTH stimulates the adrenal release of glucocorticoids (GCs), which, in turn, mobilize energy sources for the increased metabolic demands placed on the body. Acutely, this recruitment of energy sources is essential for events, such as physical exercise; however, restoration of the axis is imperative to prevent overexposure to GCs. It has been well established that chronically elevated levels of plasma GCs can lead to the development of a number of harmful pathologies, including impaired insulin signaling, central adiposity, skeletal muscle atrophy, immune suppression, and type 2 diabetes mellitus (6, 16, 24, 33).

Exercise is a potent stressor, activating the HPA axis in animals (5, 8–10, 14, 27) and in humans (13, 15, 22, 30). The effects of regular exercise on diurnal GC levels and adrenal sensitivity to ACTH secretion are unclear. Previously, a number of rodent studies investigating the effects of training on the HPA axis have employed forced exercise paradigms for their method of training (1, 25, 27, 36). Such studies have produced findings that are similar to those of investigations employing chronic stress (e.g., repeated restraint stress) as an activator of the axis (17, 32), making it difficult to delineate the effects of perceived stress from the exercise itself. Forced exercise studies have shown an enlargement of the adrenal glands, elevations in diurnal corticosterone (CORT) levels, decreased central (i.e., brain and pituitary) GC receptor (GR) density, and a reduction in the circulating ACTH-to-GC ratio (suggestive of a reduced adrenal sensitivity to ACTH). In contrast, using a voluntary running wheel design, Dishman et al. (7) found that 6 wk of endurance training in rats attenuated the ACTH response to foot shock stress, but not the GC response, suggesting that an increase in adrenal sensitivity to ACTH occurs with training. This was one of the first studies to illustrate that volitional exercise may differ from forced exercise in the adaptive responses of the HPA axis. Employing a similar voluntary exercise model, Droste and colleagues (8) recently found that rats, subjected to 4 wk of voluntary wheel running, had similar ACTH levels but higher GC levels during forced swim stress compared with previously sedentary rats. Thus, unlike with forced exercise, voluntary exercise may increase adrenal sensitivity to ACTH. In line with this hypothesis, we have previously shown that long-term wheel running (6 wk) in rats yields a normal GC response to restraint stress, despite attenuated ACTH levels (14). However, it is still unclear i) if voluntary wheel running increases adrenal sensitivity to exogenous ACTH administration; 2) if the increased adrenal sensitivity to ACTH is sustained with prolonged training; and 3) how these apparent changes in adrenal sensitivity occur.

We hypothesized that voluntary exercise training transiently increases adrenal sensitivity to ACTH by acting through molecular changes in the adrenal glands. We also hypothesized that these adrenal adaptations are due to the novel stress of exercise and that long-term training would result in the return to preexercise conditions and better control of homeostasis.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Therefore, the aim of this study was to 1) reveal the effects of short- and long-term voluntary exercise training on the acute stress response to, and recovery from, a novel cross stressor; 2) directly measure adrenal sensitivity through administration of exogenous ACTH; and 3) determine the influence of voluntary wheel running on key regulatory proteins involved in the secretion of GCs from the adrenal glands.

METHODS

Animals. Thirty-two male Sprague-Dawley rats (Charles River Laboratories, Quebec, Canada), with initial weights of 175–200 g, were equally divided into four groups consisting of two sets of sedentary (S) and two sets of exercise-trained (E) animals (n = 8 per group). In addition, a fifth set of animals (n = 6) was killed at day 0, to establish a baseline group for protein analysis. All animals had a 7-day habituation period to a 12:12-h light-dark cycle (lights on at 0800 and lights off at 2000) in a temperature (22–23°C) and humidity (50–60%) controlled room.

Design (see Fig. 1). Exercising rats were singly housed in standard rodent cages (height 36.4 cm, width 26.8 cm, depth 50 cm) for either 2 wk (E2, n = 8) or 8 wk (E8, n = 8) and were given 24-h access to a standard running wheel (Harvard Apparatus). Wheel revolutions were counted daily and multiplied by the wheel circumference (10.5 cm) to obtain daily running distances. The S groups were singly housed in similar cages for either 2 wk (S2, n = 8) or for 8 wk (S8, n = 8), but without a running wheel. All animals were given food (Ralston Purina) and water ad libitum. All experiments were approved by the York University Animal Care Committee, Toronto, Canada.

Cannulation surgery. Cannulation surgeries were performed on either the day before the introduction to the running wheels (S2 and E2 rats) or after 6 wk of treatment (S8 and E8 rats), as previously described (14). During nighttime sampling (2100, 0300, and 0600 via a tail nick bleed to obtain diurnal plasma CORT concentrations. During each sampling, ~200 μl of whole blood were collected in heparinized capillary tubes (Sarstedt, Montreal, QC, Canada) and centrifuged for 90 s, and the plasma was removed and stored at −20°C for later analysis.

Restraint stress protocol. One week following cannulation surgery, rats underwent a standardized 20-min restraint stress protocol at 0800, as previously described (14). During the restraint protocol, an initial blood sample was taken to establish baseline CORT and ACTH concentrations (time 0), after which the animal was placed in a clear, plastic, aerated restraint stress tube (Harvard Apparatus, Holliston, MA) for 20 min. Following restraint stress, the animal was removed from the restraint tube and allowed to recover in its respective cage for 95 min with no access to food or water. Throughout the experiment, blood samples were taken via the carotid artery at 0, 5, 10, 20, 30, 40, 55, 70, 85, 100, and 115 min for plasma ACTH levels and at 0, 20, 85, and 115 min for plasma CORT levels. The volume of blood obtained for analysis of CORT and ACTH was ~200 and ~500 μl, respectively. The total blood volume taken from the animals throughout the procedure was ~3.5 ml/animal (1.82 ml/h), which is below the suggested maximum volume of 2.0 ml/h (29).

ACTH challenge. Seven days after the restraint stress experiment, all animals underwent a standardized exogenous ACTH challenge to gauge adrenal sensitivity to ACTH at 0800. Following an initial baseline blood sample, a low dose of human ACTH₁–24 (75 ng/kg; Peninsula Laboratories, San Carlos, CA) was administered through the carotid artery cannula exteriorized outside of the cage, after which five blood samples were taken via the cannula at 5, 10, 15, 30, 60, and 120 min for CORT concentrations. Approximately 200 μl of blood were collected at each time point, and all blood samples were frozen at −20°C until used.

On completion of this experiment, the rats were anesthetized under inhaled isoflurane and killed by decapitation. Various tissues were collected, weighed, and stored at −80°C until subsequent analysis.

Hormone concentrations. Plasma CORT and ACTH concentrations were measured using commercially available radioimmunoassay kits (MP Biomedicals, Costa Mesa, CA). The detection limit of CORT was 0.4 ng/ml, and the inter- and intra-assay coefficients of variance were 7 and 4%, respectively. The detection limit of ACTH was 2 pg/ml, and the inter- and intra-assay coefficients of variance were 7 and 5%, respectively.

Immunoblotting. Western blotting was performed as previously described (3), with the following modifications. Seventy-five micrograms of total protein obtained from the right adrenal glands were electrophoretically resolved on a 12% SDS-polyacrylamide gel. Three
gels were run with two standards on each gel, in addition to the samples, to account for any variability found between gels. Intergel variability was <4% in all experiments. The protein was then transferred overnight at 20 V to polyvinylidene difluoride paper. Blots were blocked for 2 h using 5% BSA in Tris-buffered saline plus Tween 20 and then incubated overnight with either steroidogenic acute regulatory (StAR) antibody or melanocortin receptor 2 (MC2R) antibody at 4°C (StAR: AbCam, cat. no. ab3343, 1:1,000; MC2R: Chemicon, cat. no. AB5128, 1:1,000). Blots were then washed 5 × 10 min in Tris-buffered saline plus Tween 20 and incubated with secondary antibody for 1 h at room temperature (anti-rabbit conjugated with horseradish peroxidase; 1:5,000). Hybridization signals were visualized using the Western Lightning Chemiluminescence Reagent Plus kit (Millipore, cat. no. WBKLS0500) after exposure to Kodak X-Omat Blue X-ray film (Rochester, NY). The densitometry of each band was determined and presented relative to the basal group. GAPDH was used as a loading control.

Data analysis. For all experiments, a t-test, one-way or two-way ANOVA was performed, as appropriate, to identify significant differences in between treatment groups using Statistica 6.0 software, with \( P < 0.05 \) as the criterion. When a significant difference was observed with an ANOVA, post hoc analysis was performed using a Tukey-Kramer test to determine specific differences. Values are presented as means ± SE.

RESULTS

Anthropometry. All groups gained body weight with time; however, S rats gained weight at a faster rate than E rats \( (P < 0.001) \). As a result, S animals weighed significantly more than their matching E group by day 8 \( (+13.6 \text{ and } +17.2 \text{ g in the 2- and 8-wk groups, respectively, } P < 0.05) \). All animals demonstrated a decrease in body weight in response to the cannu-

<table>
<thead>
<tr>
<th>Time of Day</th>
<th>2-wk Treatment Group</th>
<th>8-wk Treatment Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sedentary</td>
<td>Exercise</td>
</tr>
<tr>
<td>0300</td>
<td>16.59 ± 3.37</td>
<td>35.81 ± 9.29*</td>
</tr>
<tr>
<td>1000</td>
<td>16.19 ± 4.49</td>
<td>23.57 ± 8.11</td>
</tr>
<tr>
<td>2100</td>
<td>44.73 ± 9.95</td>
<td>46.87 ± 11.50</td>
</tr>
</tbody>
</table>

Values are means ± SE in ng/ml. Diurnal corticosterone values for sedentary and exercise animals at 1 wk and 7 wk are given. Blood was sampled at lights out (2100), in the middle of the night (0300), and at lights on (1000) to assess daily diurnal corticosterone patterns. The 2-wk exercised animals had higher corticosterone values at 0300. *\( P < 0.05 \) vs. 2-wk sedentary.

Fig. 2. The response and recovery profile of ACTH and CORT in response to, and recovery from, 20-min restraint in the 2-wk (A and C) and 8-wk groups (B and D). A: E2 were found to have a higher ACTH response to restraint stress compared with S2 (2-wk sedentary rats) at the 20-min time point \( (*P < 0.01) \). B: no difference in ACTH levels between S8 (8-wk sedentary) and E8 were found at any time point. C: E2 were found to have a higher CORT response to restraint stress compared with S2 at the 5- and 10-min time points \( (*P < 0.01) \). D: no difference in CORT levels between S8 and E8 were found at any time point.
luation surgery ($P < 0.05$), as has previously been reported (14). However, following surgery, all groups continued to gain weight as expected. E animals also demonstrated decreased epididymal and perirenal fat mass compared with their S counterpart (data not shown). No difference was found in adrenal, soleus, or plantaris weight (data not shown).

Running distance. Both E2 and E8 had similar running distances during the first 2 wk (Table 1). E8 continued to progressively increase their average weekly distance ran to a maximum of 8,875 ± 2,731 m/day during week 4, where it remained until cannulation surgery during week 6. Following cannulation surgery, the average weekly running distance dropped to 3,445 ± 1,029 m/day in the E8 group, but then rebounded in these animals to that observed presurgery, averaging 5,973 ± 1,176 m in week 8.

Diurnal CORT results. After 1 wk of treatment, E demonstrated higher CORT concentrations at 0300 (Table 2, $P < 0.01$) than S animals, indicating hyperactivation of the HPA axis in the former group following lights out. No differences were found at 1000 or 2100 between E and S at week 1. At day 49, no differences were found in diurnal GC levels between S and E rats, despite the high running distances observed in the latter group.

Restraint stress experiments. To assess the effects of both short- and long-term training to a novel stressor, restraint stress protocols were carried out 8 days following surgery in all groups. The restraint stress experiments show significant differences between S and E animals at day 8, but not at day 50 (Fig. 2). E and S rats had similar basal CORT values before the restraint on day 8, suggesting that the animals had recovered from the cannulation surgery similarly, but E responded to the novel stress with a greater increase in CORT concentrations than S for the first 10 min of the restraint (Fig. 2C; $P < 0.01$). CORT levels at 20 min were also higher in E than S, but only a trend was noted for statistical significance ($P = 0.09$). S and E had similar CORT values throughout the recovery period, and values returned to baseline by ~90 min following the end of restraint in both groups. Differences in ACTH concentrations were also noted during the restraint protocol in the short-term- but not in the long-term-treated rats. In particular, on day 8 (i.e., short-term training), E responded to restraint with lower ACTH concentrations compared with S at 20 min ($P < 0.01$, Fig. 2A). No differences in ACTH were observed between the two groups during the recovery period, however. At day 50, the CORT response to, and recovery from, restraint was similar between S and E animals (Fig. 2D). Furthermore, no differences were seen in ACTH at any point during the restraint stress protocol (Fig. 2B).

ACTH challenge. To assess adrenal sensitivity to ACTH, a standardized ACTH challenge was given 1 wk following the restraint stress experiment. Similar to the restraint experiments, differences between E and S animals were seen at short-term but not at long-term treatment. In particular, on day 14, E demonstrated increased adrenal sensitivity to ACTH compared with S (Fig. 3A), with CORT concentrations higher in E than in S at 5, 10, and 15 min ($P < 0.05$). E and S had similar values after 30 min and thereafter. In contrast, no difference in ACTH sensitivity was found in the 8-wk animals at any time points (Fig. 3B).

MC2R and StAR. To help elucidate the molecular mechanisms behind increased adrenal sensitivity to ACTH with short-term training, we determined the expression of two key adrenal proteins involved in the synthesis and release of CORT in short- and long-term-treated S and E animals and in animals before the training intervention (basal rats). MC2R binds ACTH in the adrenal glands, initiating the signaling process to release CORT. A trend was noted for increased expression of MC2R in E compared with S at 2 wk and basal rats ($P = 0.08$, Fig. 4A). No difference was found between S and E groups in MC2R expression at 8 wk of treatment. StAR protein is involved in the import of cholesterol into the adrenocortical cells and is proposed to be the rate-limiting step in GC biosynthesis (2). StAR expression was significantly increased in E compared with S at 2 wk and basal rats ($P < 0.05$, Fig. 4B). No difference was found in StAR between S and E rats at 8 wk.

DISCUSSION

It is well known that acute exercise is a potent stressor that activates the HPA axis in humans and in rodents (8–10, 13–15). This study reveals that, with regular exercise, in the form of voluntary wheel running, healthy rodents initially have...
hyperactivation of the HPA axis, as evidenced by elevations in diurnal CORT levels, an enhanced CORT response to restraint stress, and an increased adrenal response to exogenous ACTH challenge. Furthermore, we confirm that, even with increased wheel running, long-term endurance-trained rats have normal diurnal HPA activity and normal response to restraint stress and ACTH challenge. We propose the mechanism(s) behind the adaptive effects of training on peripheral HPA axis activity occurs at the level of the adrenal gland, specifically through changes in ACTH sensitivity via transient increases in the ACTH receptor known as the MC2R and the rate-limiting steroidogenic protein called StAR. These novel findings suggest that short-term endurance training in untrained individuals may result in a temporary increase in GC levels (i.e., from days to weeks, depending on their adaptive capacity) and increased adrenal sensitivity to ACTH; however, sustained training is associated with normal diurnal GC activity, a restored adrenal sensitivity to ACTH, and a normal HPA responsiveness to a nonexercise stressor.

The effect of endurance training on basal (i.e., unstimulated) GC levels is somewhat controversial. Marathon runners have normal resting GC concentrations, despite higher concentrations of ACTH, suggesting that training may decrease adrenal sensitivity to ACTH (11, 37). In rodents, the effect of exercise on diurnal HPA activity and adrenal sensitivity to ACTH is also unclear. For example, compared with S animals, trained animals have increased resting stress hormones (17, 25), while others show similar resting concentrations (14, 27) or even decreased resting concentrations (4, 7, 9, 19). It is likely that the variety of training protocols and the varying training durations used, as well as the timing of blood sampling in these rodent studies, contributes to the discrepancies in findings. With respect to HPA activation, voluntary wheel running may be a better experimental model than forced exercise, such as swimming or treadmill running, as wheel running cages allow animals to exercise during their normal wake cycle (i.e., evening with lights off) and without the negative reinforcement usually required to initiate and maintain exercise intensities (i.e., foot shocks). Our voluntary wheel-running model was undertaken to remove such confounding variables and allow the measurement of exercise interventions directly on the HPA axis.

In this study, and previously (14), we show that short-term voluntary wheel running (i.e., 1–2 wk of training) elicits higher diurnal CORT patterns compared with S animals. This elevation in early morning CORT with short-term wheel running is identical to what has been observed by others using a 4-wk training paradigm in mice (9). However, we also show that, with prolonged training (lasting 8 wk in duration), the diurnal CORT pattern in E rats is similar to that observed in S animals (Table 2). In agreement with this, long-term wheel running with or without the antidepressant Tianeptine in mice is associated with lower early morning GC levels compared with S mice (10). Moreover, long-term wheel running in young hamsters, but not in old hamsters, is associated with lower basal GC levels compared with that in S hamsters, despite young hamsters running ~20 km per night (4). Thus it is clear that the HPA axis adapts with training, likely to protect the exercising animals from the negative consequences of elevated GC concentrations. Indeed, chronic elevations in HPA activity, to levels observed during the initial 2 wk of training, would likely counter some of the beneficial effects of exercise training itself by contributing to insulin resistance, immune suppression, muscle proteolysis, and mitochondrial loss (23). These findings in rodents coincide well with the observations that endurance-trained men have normal AM plasma ACTH and cortisol levels and normal 24-h urinary free cortisol levels on a sedentary day (12).

It is possible that the increased HPA activity during the first 2 wk of training is due to impaired central negative feedback. Reductions in central GR and MR levels have previously been illustrated to cause prolonged elevations in CORT following a chronic variable-stressor paradigm that included forced vigorous exercise (17). However, the same group also found no significant changes in hippocampus GR with milder exercise intensities (18). Indeed, neither long-term forced swim training (26) nor voluntary wheel running (8, 14) lowers central corticoreceptors in rodents. In fact, Droste et al. (8) found that
exercise can increase GR mRNA expression in distinct hippocampal cell layers, suggesting increased negative feedback sensitivity exists with 4 wk of wheel running in mice. In this study, and previously (14), we found no difference in the recovery of E and S animals following the restraint stress in either short- or long-term-trained animals (Fig. 2). This suggests that impaired negative feedback sensitivity is likely not the cause of HPA hyperactivity during the early stages of exercise training. Rather, we and others (8) believe that significant adaptations in the adrenal glands occur with prolonged training, such that adrenal sensitivity to ACTH is enhanced. Indeed, we clearly confirm this hypothesis here, by using a standardized ACTH challenge to show that exercising animals have increased adrenal sensitivity at 2 wk (Fig. 3). In further support of this, after short-term training, E rats responded to novel restraint stress with elevated CORT concentrations but with attenuated ACTH values compared with S rats (Fig. 2). Protein analysis of the adrenal glands showed that this transient increase in adrenal sensitivity to ACTH with short-term training is associated with increased expression of StAR and a tendency for an increased expression of the ACTH receptor MC2R (Fig. 4). These two proteins are thought to be the key determinants of adrenal sensitivity to ACTH (2). We believe that these short-term adrenal adaptations may be driven by the initial increase in HPA activity, resulting from the exercise itself or perhaps the running wheel environment. Indeed, ACTH upregulates the expression of its own receptors on adrenocortical cells in such a feed-forward manner (21, 28). In accordance with this, 2 wk of voluntary wheel running have recently been shown to increase mRNA levels of StAR (25). Alternatively, a recent study has also suggested that enhanced sympathoadrenomedullary activity, found to be associated with exercise training, may positively modulate adrenal sensitivity to ACTH (8). Considering that epinephrine has previously been demonstrated to stimulate adrenocortical steroidogenesis and secretion of GCs (8), our study proposes possible target genes on which this increased sympathoadrenomedullary input may be operating.

The return of diurnal GCs to levels observed in S rats with prolonged exercise training suggests that there is a desensitization of ACTH at the level of the adrenal gland. In line with this, we also observed that both MC2R and StAR protein levels decreased from 2 to 8 wk in the exercising rats, and that exogenous ACTH sensitivity was also reduced with prolonged training. The mechanism(s) for the reduction in ACTH sensitivity, despite continued training, is unclear. In this regard, we have previously shown that exercise initially causes hyperactivity of the HPA axis (measured by morning cortisol), but that prolonged training results in restoration of normal values, suggesting that a desensitization of the HPA axis exists with long-term training (14). It is also worth mentioning that, compared with the S2 rats, S8 rats also had an apparent decrease in adrenal sensitivity to exogenous ACTH, but no change in StAR or MC2R expression (Figs. 3 and 4). Elevations in stress reactivity in prepubertal female rats compared with postpubertal female rats have been observed by Romeo et al. (31), and these maturational changes have also been attributed to changes in adrenal sensitivity to ACTH. Further work is clearly warranted to help identify the mechanisms for altered adrenal sensitivity to ACTH in exercising and nonexercising states.

Our study has some important limitations that warrant discussion. First, because of the nature of our study design, we cannot unequivocally say that the upregulation in StAR protein was the cause of increased adrenal sensitivity early on in training. Rather, we report an association between these two variables, as others have done recently (34). Second, it is unclear why peak and basal GC levels increased and decreased, respectively, from 7 days to 42 days in the S rodents, even though cage enrichment and conditions remained constant (Table 2). It may be that rats of this age have a reduction in basal GC levels as a result of sexual maturation, as has been reported previously (31). It is also important to note that, as an alternative, S rats could have been exposed to the identical cage condition as the exercising rats but with the addition of a locked wheel, as has recently been used to study the metabolic effects of exercise cessation in rodents (20). Finally, it is important to note that the “short-term” training measurements, which included diurnal GC profiling, restraint stress, and ACTH challenge, were made over a span of 6 days to allow for a recovery from the restraint (see Fig. 1). It is possible, therefore, because of the apparent dynamic changes in the HPA axis at the start of training paradigm that the transient increase in MC2R and StAR expression, and the resultant elevated adrenal sensitivity to ACTH, might have already been returning toward baseline. The dynamic changes in HPA axis activity that occur during the initial period of training thus require careful profiling in future studies.

In conclusion, we demonstrate that exercise initially causes upregulation of HPA axis activity, resulting in increased circulating concentrations of CORT in rats, particularly during the lights-off cycle. Importantly, long-term training ameliorates this difference, resulting in a similar diurnal CORT pattern, as well as a similar stress response in E and S animals. We propose that these adaptations are found at the level of the adrenal gland, specifically with transient differences in StAR protein expression and concomitant increases in adrenal sensitivity to ACTH. Overall, the data presented here indicate that, although exercise initially causes perturbations in the HPA axis, long-term training can be performed without concern for chronic elevations in CORT concentrations and associated health detriments.

**REFERENCES**

7. Dishman RK, Bunnell BN, Youngstedt SD, Yoo HS, Mougey EH, Meyerhoff JL. Activity wheel running blunts increased plasma adreno-


