Changes in basal hypothalamo-pituitary-adrenal activity during exercise training are centrally mediated

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Submitted 14 February 2005; accepted in final form 4 July 2005

Mechanisms underlying the potential influence of endurance-type exercise training on central regulation of basal pituitary-adrenal activity and HPA response to acute exercise are unknown. Neuropeptides and corticosteroid receptors in the brain and anterior pituitary are largely responsible for stress hormone levels in the circulation. Surprisingly, the influence of exercise on these central regulators is largely unexplored. A recent study has shown that voluntary wheel running for 4 wk in mice decreases mineralocorticoid receptor (MR), but not glucocorticoid receptor (GR), binding in the hippocampus (13), suggesting that there may be reduced negative feedback inhibition of the HPA axis with training. However, the study did not investigate the time course of changes in central corticosteroid receptor expression that may occur with changes in peripheral HPA activity. Furthermore, alterations in GR expression in the hypothalamic paraventricular nucleus (PVN) and in the anterior pituitary, which are other crucial sites of glucocorticoid-induced negative feedback inhibition, were not examined.

In the present study, we 1) investigated the time course of changes in basal pituitary-adrenal activity in exercise trained rats and compared it with that of control rats that were subject to sham exercise and 2) examined whether peripheral adaptations are associated with changes in the expression of trophic factors and corticosteroid receptors in the brain and in the pituitary. We hypothesized that alteration of basal pituitary-adrenal activity by exercise training is mediated by changes in corticosteroid receptor expression in the brain and the pituitary. To test this hypothesis, rats were either swim trained or subjected to sham exercise to separate the effects of psychological stress associated with the swimming environment (i.e., immersion in water) from the effects of swimming itself. The hormonal responses to an acute treatment session as well as basal pituitary-adrenal activity were evaluated at different time points over the course of a 6-wk training period. Furthermore, neuropeptide and corticosteroid receptor gene expression in the brain and in the anterior pituitary were examined at regular intervals throughout the training period.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (150–175 g; Charles River Laboratories, Quebec, Canada) were individually housed in opaque microisolation cages in a temperature (22–23°C)- and humidity-

CONSIDERABLE RESEARCH has explored the mechanisms involved in hypothalamo-pituitary-adrenal (HPA) adaptation to various types of stress in humans and in animals. Exercise is a particular form of metabolic and physical stress that can induce HPA and sympatheo-adrenal activation in an intensity-dependent and activity-specific manner (9, 27). The effects of endurance-type exercise training on hormonal responses to a subsequent bout of exercise are controversial. In some (3, 28, 47) but not all (5, 20) studies, training has been found to blunt the HPA response to subsequent exercise. It is also unclear whether exercise training alters basal pituitary-adrenal activity, given that some studies have reported elevated basal pituitary-adrenal activity (14, 25, 43) whereas others have reported no change (15–18, 40).
controlled room. The animals were fed standard rat chow (Ralston Purina, St. Louis, MO) and water ad libitum. They were acclimatized to a 12:12-h light-dark cycle (lights on between 0700 and 1900) for 1 wk before experimental manipulation. All experiments were approved by the Animal Care Committee of the Faculty of Medicine at the University of Toronto in accordance with the Canadian Council for Animal Care.

Experimental design. Three main groups were used in the study: daily swimming (DS), sham exercise (SE), and no treatment (NT). All animals were handled daily for 4 days and habituated to the treatment environment for 5 days before the start of the experiment. Habituation involved placing the animals in shallow water at 35°C for 20 min in the same environments that they were to be treated in. DS and SE groups were divided into three subgroups based on the duration of treatment: 2, 4, or 6 wk (n = 7–10 rats/group). The NT group (n = 7) was maintained for 6 wk until death without any intervention and served as a control group for DS and SE groups at 6 wk.

Exercise and sham exercise treatments. Rats in the DS group swam individually in large plastic cylindrical tanks (water depth, 50 cm; temperature, ~35°C) for 45 min/day, 5 days/wk. During a swimming session, the animals were fitted with jackets containing pockets into which an incremental amount of small lead weights, which were equivalent to 2% of body weight during the first week (~4 g) with an additional 2% added each week, were inserted to increase the exercise intensity each week. The purpose of the additional weight was to attempt to maintain constant relative exercise intensity throughout the training period. During the same treatment periods, SE rats were placed in shallow water (depth, ~5 cm; temperature, ~35°C) for 45 min in plastic cylindrical tanks. SE rats were fitted with the same jackets as their swimming counterparts but without weights. After each treatment, the rats were towel-dried and placed back into their home cages under heating lamps. In both groups, treatment took place daily between 1400 and 1600.

Surgery. Surgeries took place at least 4 days before blood sampling for acute corticosterone (Cort) and catecholamine response measures during a treatment session. The carotid artery was catheterized as described previously (42). The catheter was flushed every other day to retain patency and maintained with a heparinized saline lock when not in use. Animals were given 3 days to recover from surgery in their home cages before resuming their respective treatments.

Acute hormone response to exercise or sham exercise treatment. Serial arterial blood samples were obtained from the rats during one of the treatment sessions (swimming or sham exercise) in the last week of their respective treatment protocols (i.e., in the 2nd, 4th, or 6th week) to establish how plasma Cort and catecholamine responses change over the course of a 6-wk training period. In the morning on the day of blood sampling, the catheters were extended outside the cages with the use of PE-50 tubing to minimize investigator interaction. The rats were not handled for 5 h before the first blood sample was drawn at 1400 (pretreatment). During the treatment session, blood samples (Cort, 0.3 ml; catecholamine, 0.5 ml) were taken at 15, 30, and 45 min after the initiation of treatment via catheters extended outside the treatment tanks. Recovery sample was taken 30 min after the conclusion of the treatment while the animals were resting in their home cages. Within 5 min of each blood sampling, red blood cells were resuspended in a volume of bovine serum albumin equal to the volume of the plasma collected and were reinfused into the animal in an attempt to maintain normal hematocrit levels.

Basal hormone levels and tissue collection. Rats were rapidly killed by decapitation upon removal from their home cages between 1000 and 1200, ~1 h after the last treatment session. Trunk blood was collected for assessment of basal plasma ACTH and Cort concentrations. The brain and the pituitary were rapidly removed and frozen (~80°C). In addition, deep red quadriceps muscles from the right leg were excised and frozen using liquid nitrogen. Plasma samples were stored at −20°C and tissues at −80°C for subsequent analysis.

Plasma hormone and catecholamine determination. Plasma ACTH and Cort concentrations were measured using commercially available radioimmunoassay kits (ICN Pharmaceuticals, Orangeburg, NY). Plasma catecholamine (Amersham Pharmacia Biotech, Piscataway, NJ) concentrations were determined using the simultaneous single-isotope derivative radioenzymatic assay technique described previously (37).

Cytochrome c oxidase enzyme activity. Cytochrome c oxidase (COX) activity in the deep red quadriceps muscle was determined as previously described (8). The enzyme activity was determined as the maximal rate of oxidation of fully reduced cytochrome c that is measured by changes in absorbance at 550 nm in a spectrophotometer (Beckman DU-64).

In situ hybridization. Coronal brain and pituitary cryosections (10 μm) were obtained through selected hypothalamic, hippocampal, and anterior pituitary regions according to stereotactic coordinates specified by Paxinos and Watson (36). The sections were thaw-mounted onto poly-L-lysine (Sigma)-coated slides, fixed for 5 min in 4% phosphate-buffered paraformaldehyde, rinsed in phosphate-buffered saline (PBS; 2 min), dehydrated in an ethanol series (5 and 1 min at 70 and 95%, respectively), and stored in 95% ethanol at 4°C until analysis.

The protocol for in situ hybridization was described in detail previously (31). In brief, previously characterized antisense MR, GR, corticotrophin-releasing hormone (CRH), arginine vasopressin (AVP), and proopiomelanocortin (POMC) oligonucleotide probes (6) were labeled using terminal deoxynucleotidyl transferase (Invitrogen Canada, Burlington, Canada) and deoxyadenosine 5’-C-[35S] triothiophosphate (1,300 Ci/mmol; Perkin-Elmer, Woodbridge, Canada) to a specific activity of 1.0 × 106 cpm/μg. Labeled probe in hybridization buffer (200 μl) was applied to each slide at a concentration of 1.0 × 103 cpm/μl, and slides were incubated overnight in moist chamber at 42.5°C. After being washed in 1 × saline sodium citrate (SSC; 20 min at room temperature and 35 min at 55°C), slides were rinsed twice with 1 × SSC and once with 0.1× SSC and were then dehydrated in 70 and 95% ethanol for 1 min each, at room temperature. Slides were then air-dried and exposed to autoradiographic films for various lengths of time (exposure times: GR; 28 days; MR; 14 days; AVP; 1 day; CRH; 35 days; POMC; 2 h) depending on the probe used and its radioactivity.

Data analysis. For in situ hybridization, brain sections were processed simultaneously for each probe to allow for direct comparisons among the treatment groups. Six to twelve sections were selected from each animal for each region to be analyzed by in situ hybridization. The sections were exposed along with 14C standards (American Radiochemical, St. Louis, MO) to ensure analysis in the linear region of the autoradiographic film. The relative optical density (ROD) of the signal on the film was quantified, after subtraction of background values, using a computerized image analysis system (Imaging Research, St. Catherines, ON, Canada). Hormone data are presented as means ± SE, and in situ hybridization data are expressed as ROD (means ± SE).

For the acute hormone response data, a three-way (group × number of weeks of treatment × time interval) mixed-design ANOVA was used. If no interaction was found, then a two-way ANOVA, with time intervals collapsed, was performed to test for group effect (i.e., DS vs. SE) and the effect of weeks of treatment within each group. For pretreatment and basal hormone levels, COX, GR, MR, CRH, AVP, and POMC mRNA, a two-way (group × number of weeks of treatment) between-groups ANOVA was used. If no interaction was found, data were decomposed using one-way ANOVA to ascertain the effect of treatment time within each group. Furthermore, one-way ANOVA was performed to compare DS, SE, and NT groups at 6 wk. Statistica software (version 6.0; Tulsa, OK) was used, with P ≤ 0.05 set as the limit for statistical significance.
RESULTS

Body weight, food intake, adrenal weight-to-body weight ratio, and COX enzyme activity. Table 1 represents the results for body weight, food intake, adrenal weight-to-body weight ratio (AW/BW), and COX activity. There was no difference in body weight between the DS and SE groups during the course of training. However, body weight increased from 2 to 6 wk for both DS and SE groups ($P < 0.01$). Compared with the NT group, DS and SE groups at 6 wk weighed significantly less ($P < 0.01$). Average daily food consumption did not change from 2 to 6 wk in either DS or SE group and was not different between the two groups. It was, however, significantly higher in the NT group compared with DS and SE groups at 6 wk ($P < 0.05$).

![Fig. 1. Pretreatment plasma corticosterone concentrations (left) and changes ($\Delta$) in plasma corticosterone concentrations from pretreatment in response to daily swimming (DS; solid bars and solid lines, $n = 5–6$) or sham exercise (SE; open bars and dashed lines, $n = 5–7$) (right) at 2 wk (top), 4 wk (middle), and 6 wk (bottom) of the treatment period. Horizontal bar indicates the duration of the treatment session. Results are expressed as means $\pm$ SE. *$P < 0.01$ vs. SE at the same time points. **$P < 0.01$ vs. 2 wk. *$P < 0.05$ vs. DS and SE at 6 wk. *$P < 0.01$ vs. DS at 6 wk.](http://ajpregu.physiology.org/)

Table 1. *Body weight, average daily food intake, adrenal weight-to-body weight ratio, and COX activity*

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<th>DS</th>
<th>SE</th>
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<td>2 Wk</td>
<td>4 Wk</td>
<td>6 Wk</td>
</tr>
<tr>
<td>$n$</td>
<td>7–10</td>
<td>7–10</td>
<td>7–10</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>314.8±6.3</td>
<td>330.3±8.5</td>
<td>369.4±18.4bc</td>
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<td>Average daily food intake, g</td>
<td>25.5±1.0</td>
<td>23.8±1.4</td>
<td>26.0±1.4</td>
</tr>
<tr>
<td>Adrenal weight-to-body weight ratio, mg/g</td>
<td>0.088±0.006</td>
<td>0.096±0.007</td>
<td>0.100±0.01</td>
</tr>
<tr>
<td>COX activity, μmol·min$^{-1}$·g$^{-1}$</td>
<td>10.8±0.7a</td>
<td>10.5±0.9a</td>
<td>12.0±0.8a</td>
</tr>
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</table>

Body weight, average daily food intake, adrenal weight-to-body weight ratio, and cytochrome c oxidase (COX) activity in the daily swimming (DS) sham exercise (SE), and no treatment (NT) groups. Results are expressed as means $\pm$ SE. *$P < 0.01$ vs. SE at the same time points. **$P < 0.01$ vs. 2 wk. *$P < 0.01$ vs. NT. *$P < 0.05$ vs. DS and SE at 6 wk. *$P < 0.01$ vs. DS at 6 wk.
In the DS group, AW/BW did not change, whereas in the SE group, AW/BW increased significantly from 2 to 4 wk ($P < 0.01$) and then declined slightly but nonsignificantly from 4 to 6 wk. AW/BW did not differ between DS and SE animals at any time point of the training period. The AW/BW ratio of the NT group was significantly lower than that of the DS group but not the SE group.

COX activity in deep red quadriceps muscle of the DS group was higher than SE group at 2, 4, and 6 wk ($P < 0.01$). COX activity in the NT group was lower than that in the DS group at 6 wk ($P < 0.01$) and was not different from that in the SE group.

**Pretreatment Cort and catecholamine concentrations.** Figures 1, 2, and 3, left, depict pretreatment plasma Cort and catecholamine concentrations that were measured 2 h before the initiation of treatment (i.e., at 1400). Pretreatment plasma Cort concentration increased from 2 to 4 wk ($P < 0.05$) but remained unchanged from 4 to 6 wk in the DS group. Pretreatment epinephrine (Epi) concentration increased from 2 to 4 wk ($P < 0.05$) and remained unchanged from 4 to 6 wk, whereas norepinephrine (NE) concentration increased from 2 to 4 wk and then returned to 2-wk levels at 6 wk (both $P < 0.05$). In the SE group, pretreatment Cort, Epi, and NE levels did not change from 2 to 6 wk.

**Cort and catecholamine responses during swimming or sham exercise.** Figure 1, right, shows the changes in plasma Cort concentration from pretreatment levels during a bout of swimming or sham exercise at 2, 4, and 6 wk. At 2 and 4 wk, DS and SE animals showed similar Cort responses. At 6 wk, the Cort response was greater than at 4 wk in the DS group. Furthermore, at 6 wk, we observed higher values in the DS group than in the SE group ($P < 0.01$). Cort response to sham exercise did not change from 2 to 6 wk.

Figures 2 and 3, right, show the changes in plasma Epi and NE concentrations, respectively, from pretreatment levels during a treatment session at 2, 4, and 6 wk. Epi and NE responses to a bout of swimming in the DS group increased from 2 to 4 wk ($P < 0.05$) and then remained unchanged from 4 to 6 wk.
On the other hand, Epi and NE responses in the SE group did not change over the 6-wk period. Moreover, the DS group had markedly higher Epi and NE responses than the SE group at 4 and 6 wk (both $P < 0.01$).

**Basal plasma ACTH and Cort concentrations.** Basal plasma ACTH concentrations are shown in Fig. 4, top. In the DS group, basal ACTH concentration initially increased from 2 to 4 wk ($P = 0.05$) and remained unchanged at 6 wk. In the SE group, basal ACTH levels did not change from 2 to 4 wk but increased from 4 to 6 wk ($P < 0.01$). Furthermore, the SE group at 6 wk had higher basal ACTH compared with the DS group (DS, $P < 0.05$; SE, $P < 0.01$).

In the DS group, basal plasma Cort concentration (Fig. 4, bottom) increased ($P < 0.01$) from 2 to 4 wk but did not change from 4 to 6 wk. There was no significant difference between the DS and NT groups at 6 wk. In contrast, in the SE group, basal Cort levels markedly increased from 4 to 6 wk ($P < 0.01$) with 6-wk levels higher than both DS ($P = 0.05$) and NT groups ($P < 0.05$).

**Corticosteroid receptor expression.** MR mRNA expression in all animals was almost exclusively localized to limbic structures in the rat brain. Because MR mRNA is expressed heterogeneously in different regions of the hippocampus (CA1/2, CA3, CA4) and dentate gyrus (DG), the regions were analyzed separately (Fig. 5). In the DS group, hippocampal MR mRNA did not change in the four regions examined over the 6-wk period. However, in the SE group, there was a significant decrease in MR mRNA in all regions examined from 4 to 6 wk. Furthermore, SE rats had lower MR mRNA than DS rats at 6 wk in all four regions. MR mRNA expression in the NT group was lower than in the DS group in the CA3 region and higher than the SE group in the DG region.

GR mRNA expression was examined in the hippocampus (Fig. 6), the hypothalamic PVN (Fig. 7), and anterior pituitary (Fig. 8). As for MR, different regions of the hippocampus and DG were analyzed separately. In the DS group, GR mRNA expression was unchanged from 2 to 6 wk in the CA1/2, CA3, and DG regions but decreased from 2 to 6 wk in the CA4 region ($P < 0.05$). In the SE group, GR mRNA levels decreased from 2 to 6 wk in the CA1/2, CA3, and CA4 regions.
of hippocampus (all $P < 0.01$) but did not change in the DG region. At 6 wk, compared with the NT group, GR mRNA expression was lower in the SE group in the CA1/2 and CA 3 regions (both $P < 0.05$) and tended to be lower in the CA4 region ($P = 0.07$).

In the PVN, GR mRNA levels in the DS group decreased from 2 to 4 wk ($P < 0.01$) and remained unchanged from 4 to 6 wk. In the SE group, GR mRNA decreased from 4 to 6 wk ($P < 0.01$). At 4 wk, SE rats had higher GR mRNA levels than DS rats ($P < 0.01$), whereas at 6 wk, SE rats had lower GR mRNA levels than DS rats ($P < 0.05$). At 6 wk, neither DS nor SE levels were significantly different from NT levels.

In the anterior pituitary, GR mRNA levels in the DS group decreased from 2 to 4 wk ($P < 0.01$) and then increased at 6 wk ($P < 0.01$). In the SE group, GR mRNA levels increased from 4 to 6 wk ($P = 0.01$). Neither DS nor SE GR mRNA levels at 6 wk were different from NT levels.

CRH, AVP, and POMC mRNA. In the DS group, CRH mRNA levels in the hypothalamic PVN increased from 2 to 4 wk ($P < 0.01$) and then decreased from 4 to 6 wk ($P < 0.01$) (Fig. 9). In the SE group, CRH mRNA levels did not change from 2 to 6 wk. CRH mRNA levels were not different among DS, SE, and NT groups at 6 wk.

In both the PVN and supraoptic nucleus regions, AVP mRNA levels did not change in either DS or SE groups over the course of training period, and the levels in these groups at 6 wk were not different from the NT levels (Table 2).

POMC levels did not change in either the DS or the SE groups (Fig. 10). At 6 wk, POMC mRNA expression in the two groups was not different from each other but was higher than in the NT group ($P < 0.05$).

**DISCUSSION**

In the present study, we examined the effect of endurance-type exercise training on the HPA responsiveness to an acute exercise and investigated whether changes in basal HPA activity during exercise training are centrally mediated. We have shown that daily swimming with increasing absolute intensity results in robust adrenocortical and sympathoadrenal responses to acute exercise even after 6 wk of training and that, at 6 wk, both DS and SE rats exhibit strong Cort responses, whereas only DS rats display a robust catecholamine response. Furthermore, we have demonstrated that exercise training leads to an initial rise in basal plasma ACTH and Cort concentrations that appear to plateau at levels that are considerably lower than those of rats exposed to the psychological stress of sham exercise (Fig. 4). Correspondingly, our molecular data indicate that a transient increase in hypothalamic CRH mRNA levels and decreases in GR gene expression in the hypothalamic PVN and in the anterior pituitary that occur with daily swimming may be the potential mechanism for the transient rise in basal pituitary-adrenal activity (see Fig. 11).
Fig. 5. Computerized images and densitometric analysis of mineralocorticoid receptor (MR) mRNA expression in the CA1/2, CA3, CA4, and dentate gyrus (DG) regions of the hippocampus at 2, 4, and 6 wk in DS (n = 6–7) and SE (n = 6–7) as well as NT (n = 6) rats after in situ hybridization. Results are expressed as means ± SE in relative optical density (ROD) units. *P < 0.05 vs. SE at 4 wk, **P < 0.05 vs. DS at 6 wk. ##P < 0.05 vs. SE at 6 wk.

Fig. 6. Densitometric analysis of glucocorticoid receptor (GR) mRNA expression in the CA1/2, CA3, CA4, and DG regions of the hippocampus at 2, 4, and 6 wk in DS (n = 6–7) and SE (n = 6–7) as well as NT (n = 6) rats after in situ hybridization. Results are expressed as means ± SE in ROD units. *P < 0.01 vs. DS at 2 wk. #P < 0.05 vs. SE at 2 wk. **P < 0.05 vs. SE at 6 wk.
Physical parameters. DS and SE rats did not show any differences in body weight, food intake, or adrenal weight. However, their body weights were significantly lower than NT rats. This finding, at least in part, can be attributed to their lower daily food intake, which is likely related to daily stress of swimming and sham exercise. As expected, COX activity was higher in the DS group than in the SE group, indicating that 6 wk of exercise training increases aerobic capacity and mitochondrial content in rat skeletal muscle (Table 1).

Acute hormonal response to exercise or sham exercise. Several studies have reported that the HPA axis adapts to exercise training such that a response to subsequent exercise of the same absolute intensity is attenuated (3, 28, 47). As such, stimulation of the HPA axis appears to be coupled to relative, rather than absolute, exercise intensity (23, 28). Furthermore, Duclos et al. (14) have demonstrated that ACTH and Cort responses following pituitary and adrenal stimulation, respectively, are not blunted in endurance-trained athletes. Our finding of robust Cort and catecholamine responses in the DS group at 6 wk compared with those at 2 and 4 wk supports the notion that the HPA and sympathoadrenal responsiveness to exercise is not attenuated when the absolute exercise intensity is progressively increased (Figs. 1–3). Interestingly, we did not observe significant catecholamine response to sham exercise. Such large differences in the catecholamine responses between the DS and SE groups indicate that strong catecholamine response to exercise may be important in meeting the metabolic demands of daily swimming. Moreover, relatively similar Cort responses between the DS and SE groups (Fig. 1) but a markedly stronger catecholamine response in the former compared with the latter group (Figs. 2 and 3) suggest a possible stressor-specific activation of the two systems whereby the sympathoadrenal system is preferentially activated by exercise over sham exercise.

Fig. 7. Computerized images and densitometric analysis of GR mRNA expression in the paraventricular nucleus (PVN) at 2, 4, and 6 wk in DS (n = 6–7) and SE (n = 6–7) as well as NT (n = 6) rats after in situ hybridization. Results are expressed as means ± SE in ROD units. *P < 0.01 vs. DS at 2 wk. #P < 0.01 vs. DS at 4 wk. **P < 0.05 vs. DS at 6 wk.

Fig. 8. Densitometric analysis of GR mRNA expression in the anterior pituitary at 2, 4, and 6 wk in DS (n = 6–7) and SE (n = 6–7) as well as NT (n = 6) rats after in situ hybridization. Results are expressed as means ± SE in ROD units. *P < 0.01 vs. DS at 2 and 6 wk. **P = 0.01 vs. SE at 2 and 4 wk.
Basal measurements of peripheral and central HPA function. The evidence regarding the effect of exercise training on basal plasma ACTH and Cort concentrations is controversial, with studies showing elevated levels (26, 43), no change (16–18, 40), or only transient increases (4, 19). In the current study, basal plasma ACTH and Cort data suggest that exercise training is associated with a modest but significant transient increase in basal adrenocortical activity that normalizes around 4–6 wk of training (Fig. 4). This initial increase in the basal pituitary-adrenal activity may be important to allow the DS rats to meet the enhanced metabolic demands of daily swimming until the animals eventually adapt to the training stimulus, whereas the eventual plateau in Cort levels around 4–6 wk may prevent potentially detrimental effects associated with chronically elevated glucocorticoid levels. These findings confirm other observations (19, 45) that elevated basal Cort levels in treadmill-trained rats at the 2nd and 4th week of training return to pretraining levels after 6 wk. On the other hand, the dramatic increase in basal pituitary-adrenal activity in SE rats at 6 wk suggests that repeated sham exercise constitutes a chronic stress to which the animals failed to adapt, as has been reported previously with repeated water immersion and restraint stress (32).

It should be noted that at 6 wk, pretreatment Cort levels in the SE group were similar to those observed in the DS group (Fig. 1), whereas their basal Cort levels were greater than those of DS rats (Fig. 4). In the DS group, pretreatment Cort levels (measured in the afternoon) were higher than basal Cort levels (measured in the morning), suggesting that normal diurnal variation in Cort secretion is maintained. On the other hand, pretreatment and basal Cort levels were similar in the SE group, indicating that repeated sham exercise elevates morning (basal) Cort release. Indeed, it has been shown that chronic/repeated stress alters basal Cort levels in rats by increasing morning (nadir), but not afternoon, Cort secretions (35).

Corticosteroid receptors in the brain influence HPA activity by altering CRH production (7, 21, 38). In the brain, the MR binds Cort with high affinity and is mainly localized in the hippocampus (12, 39, 46). MR is occupied at low Cort concentrations and therefore has been implicated in tonic regulation of basal HPA activity (2, 10). We found that exercise training does not change MR mRNA expression in any regions of the hippocampus (Fig. 5) despite considerable Cort response to daily swimming. Given that the difference between the DS and SE groups can be attributed to the effect of exercise per se, this finding suggests the possibility that exercise training prevents MR mRNA downregulation that would normally accompany other types of repeated stress, thereby maintaining robust negative feedback sensitivity to circulating glucocorticoids. This finding is in contrast to observations of Droste et al. (13), who found decreased MR binding capacity in the hippocampus of mice that ran voluntarily (~4 km/night) for 4 wk. Given the voluntary nature of the training method, it is surprising that their finding of decreased MR binding capacity in exercising rats failed to adapt, as has been reported previously with repeated water immersion and restraint stress (32).

Table 2. AVP mRNA expression in the paraventricular nucleus and the supraoptic nucleus

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<th>Treatment Groups</th>
<th>DS</th>
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<tr>
<td><strong>PVN AVP mRNA, ROD</strong></td>
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<tr>
<td>2 Wk</td>
<td>6.57±0.56*</td>
<td>7.37±0.58</td>
<td>6.51±0.41</td>
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<tr>
<td>4 Wk</td>
<td>8.50±0.40</td>
<td>8.00±0.48</td>
<td>6.43±0.55</td>
</tr>
<tr>
<td>6 Wk</td>
<td>24.7±1.9</td>
<td>25.8±0.7</td>
<td>26.0±1.5</td>
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<tr>
<td><strong>SON AVP mRNA, ROD</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>2 Wk</td>
<td>25.5±1.0</td>
<td>23.8±1.4</td>
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<tr>
<td>4 Wk</td>
<td>24.7±1.9</td>
<td>25.8±0.7</td>
<td>26.0±1.5</td>
</tr>
<tr>
<td>6 Wk</td>
<td>29.7±0.9</td>
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Arginine vasopressin (AVP) mRNA expression in the paraventricular nucleus (PVN) and the supraoptic nucleus (SON) at 2, 4, and 6 wk in the DS (n = 6–7), SE (n = 6–7), and NT (n = 6) groups. ROD, units of relative optical density. Results are expressed as means ± SE. *P < 0.01 vs. DS at the same time point.
mice parallels the decreased MR mRNA expression observed in the SE rats at 6 wk in our study and in rats exposed to other types of repeated stress (22, 33, 41). It may be that higher volumes of exercise training are associated with a decrease in central MR, whereas training with shorter exercise bouts, as was the case in our study, does not alter MR levels.

The GR in the brain and pituitary are essential in negative feedback regulation of the HPA axis, and their downregulation results in increased HPA activity (7, 21, 38). Accordingly, a decrease in GR mRNA levels in the PVN of DS rats from 2 to 4 wk (Fig. 7) may help explain the transient increases in CRH mRNA levels (Fig. 9) and in the basal pituitary-adrenal activity. Indeed, lower GRs in the PVN have been shown to decrease the sensitivity to glucocorticoid-mediated negative feedback inhibition of CRH production (11). Moreover, a temporary decrease in pituitary GR mRNA levels, and therefore in negative feedback inhibition, at 4 wk provides further molecular basis for the transient increase in the basal pituitary-adrenal activity in the DS group. The return of CRH mRNA expression to 2-wk levels at 6 wk despite unchanged PVN GR mRNA levels from 4 to 6 wk in the DS group was unexpected but may be due to enhanced inhibitory and/or decreased stimulatory afferent inputs from various sites in the brain that innervate the PVN.

Several studies have reported downregulation of corticosteroid receptors after repeated exposure to stress (22, 41). In this study, repeated sham exercise was shown to decrease MR mRNA levels in the hippocampus (Fig. 5) and GR mRNA levels in the PVN (Fig. 7) from 4 to 6 wk. Both hippocampal MR and PVN GR mRNA expression was also lower in SE rats compared with DS rats at 6 wk. These findings point to receptor downregulation due to elevated basal Cort secretion and are consistent with the results of Mizoguchi et al. (34), who found GR downregulation in the hippocampus and elevated basal Cort levels in rats subject to repeated water immersion and restraint. In line with these observations, we found that repeated sham exercise also decreases GR mRNA expression in the pyramidal neurons (CA1–4) of the hippocampus from 2 to 6 wk (Fig. 6). Together, these data suggest that repeated

Fig. 10. Densitometric analysis of proopiomelanocortin (POMC) mRNA expression in the anterior pituitary at 2, 4, and 6 wk in DS (n = 6–7) and SE (n = 6–7) as well as NT (n = 6) rats after in situ hybridization. Results are expressed as means ± SE in ROD units. *P < 0.05 vs. DS and SE at 6 wk.

Fig. 11. Schematic diagram illustrating how exercise training and sham exercise differentially alter the gene expression of corticosteroid receptors and the neuropeptides over the course of a 6-wk training period. Changes indicated are with respect to 2-wk levels. Solid arrows indicate negative feedback pathways, and minus signs represent sights of inhibitory input. Open arrowheads depict MRs, and solid arrowheads depict GRs. CORT, corticosterone; W, weeks.
sham exercise, in parallel with other nonexercise chronic stress models, leads to decreased capacity for negative feedback inhibition of the HPA axis.

Hypothalamic CRH mRNA expression in DS rats followed a trend similar to that of basal plasma ACTH and Cort levels, suggesting the possibility that exercise training normalizes initially elevated basal pituitary-adrenal activity by attenuating the central drive to the HPA axis (Fig. 9). Although some studies have suggested that AVP predominantly stimulates the HPA axis during a period of chronic or repeated stress (24, 29, 30), AVP mRNA levels, measured primarily in the magnocellular PVN, were not altered by daily swimming in our study (Table 2). When parvocellular and magnocellular AVP mRNA levels were analyzed separately, there was no difference between the DS and SE groups with respect to treatment time or between the groups (data not shown). Therefore, we cannot rule out the possibility that CRH is preferentially used as the primary stimulus for pituitary-adrenal activation during exercise training. Interestingly, POMC mRNA levels did not change in the anterior pituitary despite the alterations in the HPA axis associated with exercise. Increases in pituitary-adrenal activity without accompanying changes in POMC mRNA levels have been reported previously and may be explained by high reserves of POMC and ACTH stored in corticotropes (1, 6, 44). With such an abundant reservoir of the ACTH precursor, an increase in CRH activity, while increasing ACTH secretion, may not produce any changes in POMC mRNA levels in the pituitary (1).

On the basis of evidence that activation of MRs and GRs in the brain suppresses HPA activity through inhibition of CRH neuron (7, 21, 38), decreased MR and GR mRNA expression in SE rats would be expected to increase CRH mRNA and, correspondingly, POMC mRNA, at 6 wk. Surprisingly, we did not observe any changes in CRH or POMC mRNA levels during the 6 wk of sham exercise. Given such a dynamic nature of the HPA axis, we speculate that compensatory mechanism(s) to offset stimulatory effects of MR and GR mRNA downregulation on CRH and, therefore, POMC production occurred in the hypothalamus and/or pituitary in an attempt to normalize elevated pituitary-adrenal activity (Fig. 11). The increase in pituitary GR mRNA to normal (NT) levels that we observed at 6 wk may be one way in which this is achieved. (Fig. 8).

In conclusion, in the present study, we have shown that daily swimming with increasing absolute intensity results in robust adrenocortical and sympathetic adrenal responses to acute exercise even after 6 wk of training in rats. In addition, our results show that, during an acute swimming or sham exercise at 6 wk, both daily swimming and sham exercise rats exhibit strong corticosterone responses, whereas only daily swimming rats display a robust catecholamine response, suggesting that the sympathoadrenal system is preferentially activated by exercise. We also have demonstrated that exercise training results in a transient increase followed by normalization of basal adrenocortical activity. Such changes may be explained, at least in part, by transient decreases in GR mRNA expression in the PVN and in the anterior pituitary and by a transient increase in CRH mRNA expression. Our results reveal for the first time how the effects of endurance-type exercise training, as a chronic physical and metabolic stress, may be regulated at the molecular level in the brain and in the pituitary. Furthermore, these findings point to potential benefits of regular exercise, which facilitates maintenance of robust adrenocortical response to an acute exercise without the negative consequences associated with chronically elevated HPA activity.

ACKNOWLEDGMENTS

We thank Elena Burdett for excellent technical assistance.

GRANTS

E. Park is a recipient of the Ontario Graduate Scholarship and the York Graduate Scholarship. O. Chan is a recipient of the Canadian Institutes of Health Research Doctoral Research Award. This work was generously supported by research grants from the Canadian Institutes of Health Research (to M. Vranic, S. G. Matthews, and M. C. Riddell), the Juvenile Diabetes Foundation International (to M. Vranic, and S. G. Matthews, and M. C. Riddell), and the National Sciences and Engineering Research Council of Canada (to M. C. Riddell).

REFERENCES

EXERCISE TRAINING AND CENTRAL HPA ADAPTATION


