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Effects of Long-Term Voluntary Exercise on the Mouse Hypothalamic-Pituitary-Adrenocortical Axis

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We studied the effects of long-term (*i.e.* 4 wk) voluntary exercise on the hypothalamic-pituitary-adrenocortical (HPA) axis in male mice. Voluntary exercise was provided by giving mice access to a running wheel, in which they indeed ran for about 4 km/d. Exercising mice showed similar body weights as control animals but presented less abdominal fat, lighter thymuses, and heavier adrenal glands. Exercise resulted in asymmetric structural changes in the adrenal glands. Whereas control mice had larger left than right adrenals, this condition was abolished in exercising animals, mainly because of enlargement of the right adrenal cortex. Tyrosine hydroxylase mRNA expression in the adrenal medullas of exercising mice was increased. In exercising mice, early-morning baseline plasma ACTH levels were decreased, whereas plasma corticosterone levels at the start of the dark phase were twice as high as those in control animals. To forced swimming and restraint stress, exercising mice responded with higher corticosterone levels than those of the control animals but with similar ACTH levels. However, if exposed to a novel environment, then exercising mice presented decreased ACTH responses. Interestingly, exercising mice showed a decreased corticosterone response to novelty only when the novel environment contained a functioning running wheel. Glucocorticoid receptor levels were unchanged, whereas mineralocorticoid receptor levels were decreased, in hippocampus of exercising animals. Corticotropin-releasing factor mRNA levels in the paraventricular nucleus were lower in exercising mice. Thus, voluntary exercise results in complex, adaptive changes at various levels within the HPA axis as well as in sympathoadrenomedullary and limbic/neocortical afferent control mechanisms. These changes seem to underlie the differential responsiveness of the HPA axis to physical *vs.* emotional challenges. (*Endocrinology* 144: 3012–3023, 2003)

EVIDENCE HAS BEEN accumulating that physical exercise has positive effects on a variety of biological systems such as body composition, the cardiovascular system, the immune system, and also the brain. With regard to body composition, the amount of peritoneal and perirenal adipose tissue, which is indicative of the risk for cardiovascular pathology, is decreased in subjects performing regular physical activity (1, 2). A decreased heart rate, an enhanced oxidative capacity, and a decreased blood pressure, have been observed under resting conditions in exercised rats (3) and humans (4). Moderate training intensity also has been shown to enhance immune system function and to increase resistance to infections (5, 6).

At the level of the brain, voluntary exercise (by allowing access to a running wheel) has been shown, in rodents, to result in an enhanced performance in spatial learning and memory tasks (7). Moreover, it has been shown, in rats and mice, that voluntary exercise increases neurogenesis in the dentate gyrus of the hippocampus, which is thought to be the result of an enhanced action of growth factors (*e.g.* IGF-1, brain-derived neurotrophic factor) in the brain (8–12). In contrast to these stimulatory effects of exercise, a decrease in neurogenesis has been observed after exposing rats or mice to psychological stressors such as forced swimming or predators (9, 10). Thus, it seems that regular physical exercise has effects, on various biological systems, that generally can be valued as positive. It has been suggested that animals, including humans, show improved coping with stressful events after regular performance of moderate physical exercise (13–15). The here-mentioned form of regular exercise is not to be mixed up with the high-demand endurance training (*e.g.* marathon running) in humans. Voluntary exercise yields many positive biological effects, whereas endurance training, because of its excessive (eccentric) physical demand, has been found to cause injuries (16, 17), reproductive disturbances (16, 18), impaired immunity (19), accelerated wear of the movement apparatus (16, 20), and chronic stress-like changes in the hypothalamic-pituitary-adrenocortical (HPA) axis (21–23).

The observation that voluntary exercise exerts positive effects at the cellular, physiological, and behavioral levels prompted us to investigate whether this would also involve the HPA axis. This because the HPA axis is a neuroendocrine system highly involved in the coping response to metabolic and stressful challenges (for review, see Refs. 24–26). Surprisingly, the HPA axis changes that occur after voluntary exercise have not been investigated in a comprehensive manner. Here, we show that long-term voluntary exercise indeed induces extensive changes in the mouse HPA axis, under baseline conditions as well as in response to different stressful challenges.

Abbreviations: AVP, Assays for vasopressin; CBG, corticosterone-binding globulin; CRF, corticotropin-releasing factor; DTT, dithiothreitol; GR, glucocorticoid receptor; HPA, hypothalamic-pituitary-adrenocortical; MR, mineralocorticoid receptor; nt, nucleotide(s); PVN, paraventricular nucleus; RT, room temperature; TH, tyrosine hydroxylase.

Materials and Methods

Animals

Male C57BL/6N mice (age at arrival, 10–12 wk; Charles River Laboratories, Inc., Sulzfeld, Germany) were singly housed in Macrolon type III cages (43 cm × 24 cm × 15 cm, l × b × h) under standard lighting

(12-h light, 12-h dark cycle, lights on at 0600 h), temperature (22–23 C), and humidity (50–60%) conditions. Food and water were available *ad libitum*.

Voluntary exercise paradigm

After habituation to the housing conditions for 5 d, the experimental group was allowed free access to a running wheel (diameter, 14 cm), in their home cages, for a period of 4 wk. Using an infrared video camera and a wheel-turning counting system, it was observed that the mice were mainly running in the wheel during the dark phase of the diurnal cycle (26a). The mice ran approximately 4 km per night, which is in agreement with other reports (27), although strain differences have been observed (28). This running performance was reached within a few days after providing the wheels to the animals (data not shown). The housing of the sedentary (*i.e.* control) animals remained unchanged. All animal experiments were approved by the government of Bavaria, Germany.

Physical parameters

The weight of the animals and food and water intake were determined weekly. After the experimental period, the animals were killed, and the abdominal (*i.e.* peritoneal + perirenal) adipose tissue, thymus, and adrenal glands were weighed.

In a separate experiment, the adrenal glands were quickly frozen on dry ice, cut into 12-μm cross-sections (the shape of the mouse adrenal is close to ellipsoid) in a cryostate, and mounted on slides (Superfrost, Menzel-Gläser, Merck & Co., Inc. Eurolab GmbH, Ismaning, Germany) previously coated with poly-L-lysine (Sigma, Deisenhofen, Germany). The adrenal sections were stored at –20 C until staining. Before staining, the sections were fixed in a modified Carnoy's solution (60% ethanol/40% glacial acetic acid) in which the glacial acetic acid is known to prevent shrinkage of the sections by the ethanol. Next, the sections were stained with Mayer's Hemalum solution and counterstained with 0.1% Eosin Y (Carl Roth GmbH, Karlsruhe, Germany). After dyeing, the sections were dehydrated and cleared in ethanol, mounted in Rotihistokit (Carl Roth GmbH), and coverslipped. For the quantitative analysis of area measures of the complete adrenal section and the separate medullar and cortical parts, we used a video image analysis system (Optimas 5.2; Media Cybernetics, Bothell, WA). Therefore, we selected 5–10 sections of each adrenal gland (from 9–10 mice per experimental group) stemming from the middle part of the gland, where it presents a virtually constant diameter over at least 20 sections (see also percent variance in the measurements given below). Digital images were recorded using a charge-coupled device video camera (XC-77CE, Sony) connected to a Carl Zeiss (Jena, Germany)/Axioplan microscope. The images were stored on a computer, and the area measures were determined using the Optimas software. The area of the complete adrenal cross-section and that of the medullar part were determined by direct measurement, whereas the area of the cortical part was estimated by subtraction of the medullar area from the total adrenal area. The mean percent variance in the measurements of the total, cortical, and medullar areas was 3.4 ± 0.3, 4.8 ± 0.3, and 7.7 ± 0.7 (mean ± SEM, n = 30 adrenals of 15 mice). These numbers indicate that the variance in the measurements of individual sections of an adrenal is rather low. Areas are expressed as the number of square pixels. Areas determined for the two adrenals of an animal are presented separately for the left and the right adrenal, as well as being added to obtain one value for the two adrenals together for total, cortical, and medullar area per animal. Subsequently, mean values ± SEM were calculated (n = 9–10 per experimental group).

Neuroendocrine experiments

All experiments, except for the circadian rhythm, were performed between 0700–1100 h. For the assessment of the circadian rhythm in plasma ACTH, corticosterone and corticosterone-binding globulin (CBG), mice were killed at either 0800 h, 1800 h (just before lights-off), or 2400 h. Individual mice were quickly anesthetized (<15 sec) in a glass jar containing saturated isoflurane (Curamed, Karlsruhe, Germany) vapor, after which the animals were decapitated immediately. Trunk blood was collected in ice-chilled EDTA-coated tubes (1.5 ml) containing 25 μg aprotinin (Trasylol; Bayer Corp., Leverkusen, Germany).

For determination of stress-induced plasma ACTH and corticosterone concentrations, the mice were either exposed to a novel environment or restraint or submitted to a forced swimming procedure. For the novel environment exposure, control or running-wheel mice were placed singly in new cages, containing clean sawdust, for 30 min, after which they were quickly anesthetized and decapitated, and trunk blood was collected as outlined above. As an extra variable, half of the control and running-wheel mice were provided additionally with a clean running wheel in the new cage. Restraint stress was achieved by putting the animals, for 30 min, in a clear plastic 50-ml Falcon-like tube (Greiner, Frickenhausen, Germany) with a diameter of 3 cm and containing ventilation holes. For forced swimming, the mice were placed, for 10 min, in a glass beaker containing water at 25 C. The water depth was 11.5 cm, not allowing the mice to touch the bottom of the glass with their paws and tail. After completion of the restraining or swimming procedure, the mice were quickly anesthetized and decapitated, and trunk blood was collected as described above.

Plasma samples for ACTH and corticosterone measurement by RIA (ICN Biomedicals, Inc., Costa Mesa, CA) were stored at –80 C and –20 C, respectively. The inter- and intraassay coefficients of variance for ACTH were 7% and 5%, respectively, with a detection limit of 2 pg/ml. For corticosterone, the inter- and intraassay coefficients of variance were 7% and 4%, respectively, with a detection limit of 0.4 ng/ml.

In situ hybridization histochemistry

Under early-morning resting conditions, mice were killed as described above. The whole brains were quickly removed, snap-frozen in isopentane at −40 C, and deep-frozen in dry ice. Twelve-micron-thick cryostate sections of the hypothalamic paraventricular nucleus (PVN; from bregma –0.70 mm to bregma –0.22 mm) were cut and mounted on slides (Super frost plus, Carl Roth GmbH). Also the adrenal glands were collected, frozen on dry ice, and cut into 12-micron sections in a cryostate.

The *in situ* hybridization assays for vasopressin (AVP) and oxytocin mRNA in the PVN and tyrosine hydroxylase (TH) mRNA in the adrenal medulla were carried out using oligonucleotide probes. The nucleotide sequence of the probes was: for AVP, 5′- GCT CAG GAA ACA AGC GGA GAG CGT AGT GTT TAG CAT CCT GGC GAG CAT -3′ [nucleotides (nt) 1493–1540 of the mouse AVP gene sequence] (29); for oxytocin, 5′- CAA GCA GGC AGC AAG CGA GAC TGG GGC AGG CCA TGG CGA TGG TGC TCA -3′ (nt 1072–1119 of the mouse oxytocin gene sequence) (29, 30); and for TH, 5′- TCA ATG GCC AGG GTG TAC GGG TCA AAC TTC ACA GAG AAT GGG CGC TGG -3′ (nt 1351–1398 of the rat TH mRNA sequence) (31). For each oligonucleotide probe, sections of all experimental groups were run in the same assay under identical conditions. Briefly, the synthesized oligonucleotides were labeled at the 3′-end with α[^{35}S]-deoxy-ATP (NEN Life Science Products, Boston, MA) using terminal deoxynucleotidyl transferase (Roche Diagnostics, Mannheim, Germany). Radiolabeled probe (10^6 cpm/200 μl/slide) was diluted into hybridization buffer [consisting of 1× Denhardt's solution, 0.25 mg/ml yeast transfer RNA, 0.5 mg/ml salmon sperm DNA, 10% dextran sulfate, 10 mM dithiothreitol (DTT) and 50% formamide (all from Sigma)] applied to the slides and incubated for 20 h at 45 C. After hybridization, the slides were washed four times in 1× SSC (150 mM NaCl, 15 mM sodium citrate, pH 7.0) at 45 C for 15 min, dehydrated in ethanol, air-dried, and exposed to autoradiography film (Biomax MR-1; Eastman Kodak Co., Rochester, NY) for 3 d.

The *in situ* hybridization histochemistry for detection of corticotropin-releasing factor (CRF) mRNA was performed using ^{35}S-labeled antisense cRNA probes. Techniques for probe synthesis, hybridization, and autoradiographic localization of mRNA signal were adapted from Simmons *et al.* (32). Briefly, the CRF probe (a gift from W. Wurst, Max Planck Institute of Psychiatry, Munich, Germany), comprising a 356-bp *Eco*R1 fragment subcloned into a pCRII-TOPO plasmid, was linearized with *Xba*I (for generation of the antisense probe; Roche Diagnostics) or BamH I (sense probe; Roche Diagnostics). The antisense CRF cRNA probe was generated using SP6 polymerase (Roche Diagnostics), whereas the sense probe was generated by using T7 polymerase (Roche Diagnostics). The used radiolabel was α[^{35}S]-uridine 5′-triphosphate (Hartmann Analytics, Braunschweig, Germany). Shortly before hybridization, the sections were air-dried at room temperature (RT), fixed in 4% formaldehyde for 10 min at RT, washed three times in PBS, and then acetylated in 0.25% acetic anhydride in 0.1 M triethanolamine for 10 min

at RT. Sections were dehydrated in serial ethanol solutions, defatted in chloroform, and allowed to dry. Subsequently, the sections were hybridized overnight, at 58 C, with a solution containing 50% formamide (Sigma), 20 mM Tris-HCl (pH 8.0) (Sigma), 300 mM NaCl (Merck KGaA, Darmstadt, Germany), 5 mM EDTA (Sigma), 10% dextran sulfate (Sigma), 0.02% Ficoll 400 (Sigma), 0.02% polyvinylpyrrolidone (Sigma), 0.02% BSA (Sigma), 0.5 mg/ml tRNA (Roche Diagnostics), 200 mM DTT (Sigma), and the ^{35}S-labeled CRF cRNA probe (5×10^6 cpm/μl), after which they were treated with 20 μg/ml ribonuclease A for 20 min at 37 C. Next, the sections were washed in 2× SSC (15 mM NaCl, 1.5 mM sodium citrate, pH 8.0)/1 mM DTT for 10 min at RT, 1× SSC/1 mM DTT for 10 min, 0.5× SSC/1 mM DTT for 10 min, twice in 0.1× SSC/1 mM DTT at 64 C for 30 min, and finally (for 10 min) in 0.1× SSC. Sections were then dehydrated in serial ethanol/ammonium acetate solutions, air-dried, and exposed to audioradiography film (Biomax MR-1, Eastman Kodak Co.) for 2–4 d.

Optical densitometry

Representative audioradiograph images of the hypothalamic PVN were digitally recorded using a charge-coupled device video camera (XC-77CE, Sony). Semiquantitative analyses of mRNA expression was performed blind using a densitometric video image analysis system (Optimas 5.2). The optical density (gray values, expressed as arbitrary units; resolution, 256 levels) of an area encompassing the PVN was determined, and the background (measured just apart from the PVN in an area containing no apparent hybridization signal) was subtracted. From each animal, at least three sections of a representative assay were analyzed. Each assay was repeated at least three times. The optical density data are presented as nett gray values (mean ± SEM of 6–8 mice/group).

With regard to the determination of TH mRNA expression in the adrenal medulla, only sections stemming from the middle part of the adrenal medulla were used for the *in situ* hybridization assays. Three assays were conducted on adrenal sections of 10 control and 10 exercising mice. In each assay, three sections per adrenal gland were used. Autoradiograms of all sections were densitometrically analyzed, providing a mean value per adrenal medulla. Here, data are presented of one representative assay. The other two assays provided similar data.

Semiquantitative analysis of the TH mRNA expression level in the adrenal medulla was performed in a similar way, as described for analysis of mRNA expression in the PVN. The optical density (gray values, expressed as arbitrary units; resolution, 256 levels) of the area encompassing the complete adrenal medulla was determined, and the background (measured just outside the adrenal gland) was subtracted. However, because the area of the adrenal medulla was different between the experimental groups and between the left and right adrenal gland, we determined an integrated optical density of TH mRNA expression by multiplying the optical density value with the respective measure of the medullar area (area expressed as square pixels/1000). Thus, the unit of integrated optical density is: nett gray value × square pixels/1000.

Surgery, tissue collection, and [^{3}H]-ligand binding assay for measurement of corticosteroid receptors and CBG

For corticosteroid binding measurement, mice were bilaterally adrenalectomized, under isoflurane (Curamed) anesthesia, to deplete their bodies of endogenous corticosteroids. After surgery, the animals were given 0.9% NaCl in the drinking water. One day after adrenalectomy, mice were quickly anesthetized with isoflurane (Curamed) and killed by decapitation. Trunk blood was collected in prechilled EDTA-coated tubes, and the plasma was checked for the absence of endogenous corticosterone, by RIA (ICN Biomedicals, Inc.). Preliminary experiments had shown that plasma corticosterone concentrations had to be less than 2.5 ng/ml, as measured in our RIA, to reach full availability of corticosteroid receptor binding by [^{3}H]-ligands (Droste, S. K., and J. M. H. M. Reul, unpublished observations). Immediately after decapitation, the brain and the pituitary were rapidly removed from the skull. Subsequently, the hippocampus tissues were dissected from the brain. Dissected tissues were instantaneously frozen in liquid nitrogen and stored at −80 C until corticosteroid receptor assay.

Basically, the receptor assays were conducted as described previously (33). Because of the small tissue size, the receptor binding capacity in individual hippocampus tissues could be determined only at a single [^{3}H]-ligand concentration (*i.e.* 10 nM achieving 90–95% saturation of binding, measurements conducted in triplicates). Tissues were homogenized in ice-cold 5 mM Tris-HCl (pH 7.4) containing 5% glycerol, 10 mM sodium molybdate, 1 mM EDTA, and 2 mM β-mercaptoethanol, using a homogenizing tube with a plastic pestle. The homogenate was centrifuged at 13,000 × *g* for 60 min at 0–2 C to obtain cytosol (*i.e.* supernatant fraction). All reagents used were analytic grade. Aliquots of cytosol (100 μl) were incubated with a single 10-nM concentration (total vol, 150 μl) of [^{3}H]-labeled steroids. Total binding to soluble macromolecules was determined with [^{3}H]-aldosterone (87–94 Ci/mmol; NEN Life Science Products, Dreieich, Germany) or with [^{3}H]-dexamethasone (85–106 Ci/mmol; Amersham Biosciences Europe GmbH, Freiburg, Germany). For measurement of the mineralocorticoid receptor (MR), total binding was assessed by incubating the cytosol with [^{3}H]-aldosterone in the presence of a 100-fold excess of the specific glucocorticoid RU 28362 [11β,17β-dihydroxy-6-methyl-17α-(1-propionyl)androsta-1,4,6-triene-3-one]. Unlabeled RU 28362 was included to prevent [^{3}H]-aldosterone from binding to the glucocorticoid receptor (GR), so that only binding of this [^{3}H]-ligand to MR was measured. Nonspecific binding was determined in parallel incubations containing a 1000-fold excess of corticosterone in addition to cytosol and [^{3}H]-aldosterone. In this manner, the MR concentration could be directly measured. Total binding of GR was determined by incubating cytosol with [^{3}H]-dexamethasone. Because [^{3}H]-dexamethasone also displays considerable affinity for MR (25), the fraction of [^{3}H]-dexamethasone binding to MR was estimated by including a 100-fold excess of RU28362 in parallel incubation tubes. This fraction was subtracted from the total [^{3}H]-dexamethasone binding. Nonspecific binding was determined in parallel incubations containing a 1000-fold excess of dexamethasone in addition to cytosol and [^{3}H]-dexamethasone. Subtraction of the nonspecific binding from the residual [^{3}H]-dexamethasone binding yielded the specific GR binding.

After incubation for 20–24 h at 0–2 C, bound and free [^{3}H]-steroid were separated by Sephadex LH-20 (Amersham Biosciences Europe GmbH) gel filtration (100 μl cytosol-[^{3}H]-steroid mixture were applied to the LH-20 columns), and radioactivity was measured in a liquid scintillation counter. The protein concentration was determined by the method of Lowry *et al.* (34), with BSA as a standard. The binding data were expressed as femtomoles per milligram of protein.

Plasma CBG levels were determined by [^{3}H]-corticosterone (specific activity, 70 Ci/mmol; Amersham) binding, as described previously (35). Therefore, plasma of intact control and exercised mice (killed at 0800, 1800, or 2400 h) was diluted 10-fold with the above-mentioned Tris-HCl buffer also used for the corticosteroid receptor binding assay. Binding of [^{3}H]-corticosterone was determined at a single more-than-95%-saturating concentration of 40 nM. With regard to the procedure of incubation, separation of bound and free, and measurement of radioactivity, the same was used as that for the corticosteroid receptor binding assay. The protein concentration was determined also by the method of Lowry *et al.* (34). For the calculation of the total number of plasma CBG binding sites, the specific activity of [^{3}H]-corticosterone was adjusted because of the presence of endogenous corticosterone in the plasma samples. The concentration of endogenous corticosterone was measured by RIA (see above).

Statistical analysis

The data on food and water intake were tested for statistically significant differences, by two-way ANOVA with repeated measures followed, in appropriate cases, by *post hoc* tests with contrasts. The experimental data on the physical parameters, hormone levels, mRNA levels, receptor levels, and plasma CBG concentrations were tested with Student's *t* test or with two-way ANOVA followed, in appropriate cases, by *post hoc* tests with contrasts. $P < 0.05$ was accepted as the level of significance. For all *post hoc* tests with contrasts, the level of significance was reduced according to the Bonferroni procedure, to keep the probability of a type 1 error less than 5%.

Results

Physical parameters

Figure 1 shows a time course of the food and water intake of control and exercising mice over the 4-wk period. Food consumption was not different between the experimental groups [effect of exercise, F(1, 8) = 1.9, $P > 0.05$; Fig. 1A]. However, the exercising mice showed an increased water intake [effect of exercise, F(1, 8) = 9.4, $P < 0.05$; Fig. 1B].

As shown in Fig. 2A, both groups of mice gained weight over the 4-wk time period [effect of time, F(6, 108) = 79.2, $P < 0.0001$], but there was no difference between the groups [effect of exercise, F(1, 8) = 1.1, $P > 0.05$; interaction time × exercise, F(6, 108) = 0.7, $P > 0.05$]. In another exercise experiment, in which mice were allowed to exercise for 12 wk also, no significant effect on body weight was observed (data not shown). By the end of the 4-wk experimental period, the exercising mice had much less (−45%) abdominal fat than the control mice (Fig. 2B), which was confirmed by nuclear magnetic resonance imaging (data not shown). Gross body inspection revealed that exercising mice presented more muscle mass, which, as indicated by the much more intense red color, also showed a higher blood perfusion.

As shown in Fig. 3, A and B, 4 wk of wheel running resulted in a decrease of thymus weight, whereas the adrenal weight had increased significantly. Together, the thymus and adrenal weight data indicate that the activity of the HPA axis was elevated during the experimental period (36).

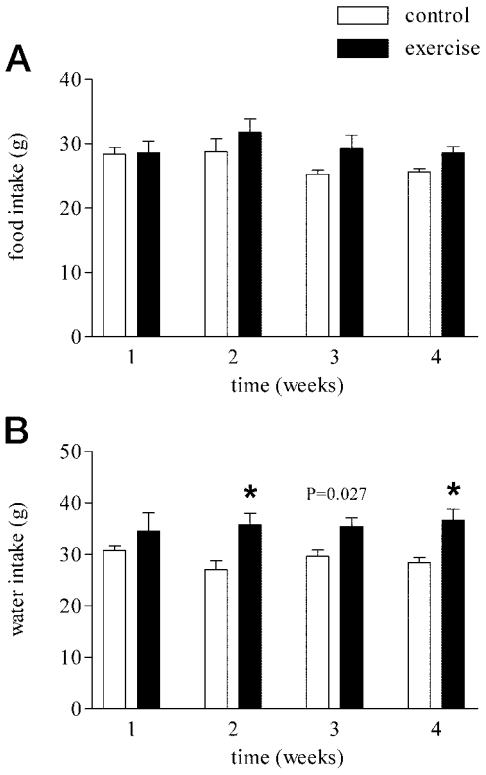

FIG. 1. Comparison of (A) food intake and (B) water consumption (means ± SEM) in exercising (n = 5) and control (n = 5) mice over a time period of 4 wk. There were no significant differences, between groups, in food intake. The water intake was significantly higher in the exercising mice from the second week onward. For ANOVA analysis, see text. *, Significantly different from control mice at the respective time point (*post hoc* tests with contrasts).

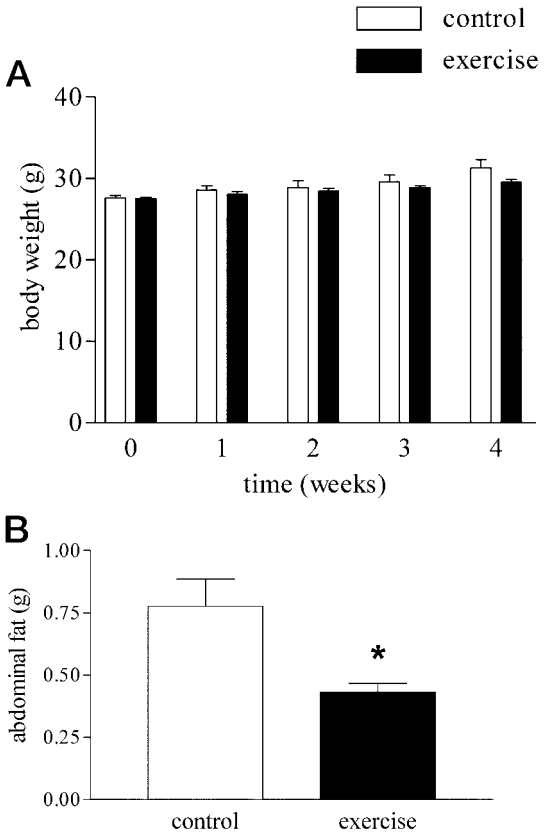

FIG. 2. Changes in (A) body weight (time course; control, n = 15; voluntary exercise, n = 15) and (B) abdominal fat tissue weight (voluntary exercise, n = 5; control, n = 5) in exercising and control mice over a 4-wk time period. Abdominal fat tissue weights were determined at the end of this time period. Data are expressed as means ± SEM. The exercising mice had significantly less abdominal fat than the control animals ($P < 0.01$, Student's *t* test).

To obtain insight into whether, after 4 wk of voluntary exercise, changes had occurred in the composition of the adrenal glands (*i.e.* cortex *vs.* medulla), area of the cortical and medullar part in adrenal cross-sections was measured (Fig. 4). Initially, we determined the entire area of cortical and medullar cross-sections of the two adrenals together, for each mouse (Fig. 4A, *Entire: Left + Right*) for sake of presenting an entire cortical and medullar capacity. This analysis showed that the entire area of the cortex is significantly increased in exercising mice (Student's *t* test, $P < 0.05$), which also almost resulted in an increased entire adrenal area (Fig. 4A, *total*). Next, we analyzed the two adrenals separately (see Fig. 4B, *Left* and *Right*). For the total area, overall, the area of the left adrenal was significantly larger than that of the right one [effect of *Left vs. Right*, F(1, 34) = 19.5, $P < 0.0001$; Fig. 4B), which was attributable both to a larger cortex (F(1, 34) = 14.9, $P < 0.0001$) and a larger medulla (F(1, 34) = 20.1, $P < 0.0001$]. Four weeks of voluntary exercise abolished the difference between the left and right total adrenal cross-sections, which was the result of a selective increase in the size of the right adrenal cortex [interaction between left/right and exercise, F (1, 34) = 4.4, $P < 0.05$; effect of exercise, F (1, 34) = 6.3, $P <$

0.05; *post hoc* tests with contrasts, significant difference between the right cortices of exercising *vs.* control mice; left cortices in exercising *vs.* control mice, not significantly different]. In addition, the difference between the left and right adrenal medulla was lost after the long-term exercise. Thus, although direct *post hoc* comparisons did not reveal a significant increase in right adrenal medulla after exercise, indirectly the absence of a left-right difference can be valued as a small increase in the size of this tissue in the exercising animals. No effects of voluntary exercise were observed in the left adrenal gland (Fig. 4B).

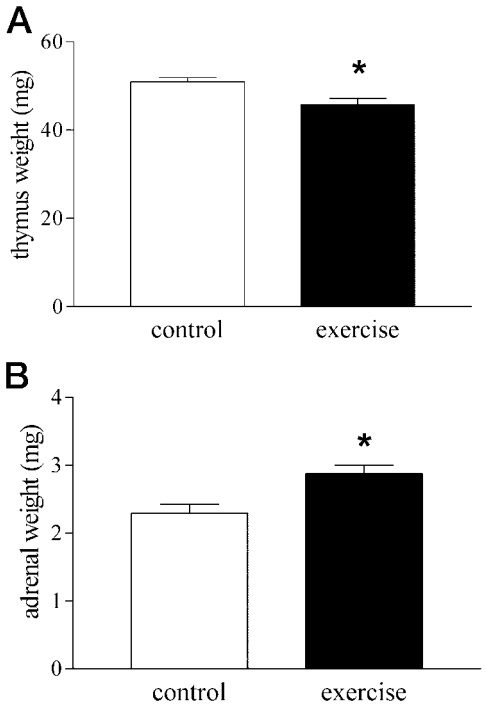

FIG. 3. Comparison of (A) thymus weight (control, n = 20; voluntary exercise, n = 19) and (B) adrenal weight (*i.e.* total weight of left + right adrenal gland; control, n = 24; voluntary exercise, n = 29) in 4-wk exercising and control mice (means ± SEM). The exercising animals had a significantly lower thymus weight ($P < 0.05$, Student's *t* test) and a significantly higher adrenal weight ($P < 0.05$, Student's *t* test) after the exercising period.

TH mRNA expression in the adrenal medulla

Because ACTH and corticosterone responses in exercising mice were not always corresponding in magnitude (see below), TH mRNA expression in the adrenal medulla was measured as an index for sympathoadrenomedullary activity (37). This activity is a well-known modulator of the sensitivity of the adrenal cortex for ACTH (38). Exercise evoked an overall increase in TH mRNA expression in the adrenal medulla [Fig. 5; effect of exercise, $F(1, 36) = 34.9$, $P < 0.0001$]. In addition, we found overall higher TH mRNA levels in the left adrenal medulla than in the right one [left *vs.* right, $F(1, 36) = 67.6$, $P < 0.0001$]. Indeed, in data of control mice, *post hoc* tests with contrasts revealed significantly higher TH mRNA levels in the left adrenal medulla than in the right one. In exercising mice, the difference in TH mRNA expression between the left and the right medulla was smaller but still statistically significant (*post hoc* test with contrasts; Fig. 5). Long-term voluntary exercise exerted a higher impact on TH mRNA levels in the right adrenal medulla than in the left one [interaction between "effect of exercise" and "left *vs.* right," $F(1, 36) = 7.26$, $P = 0.011$]. Moreover, as shown by *post hoc* tests with contrasts, a significant increase in TH mRNA levels was only observed in the right medulla after exercise.

Neuroendocrine experiments

Figure 6 shows the circadian variation in plasma ACTH, corticosterone, and CBG levels in control mice and in mice after 4 wk of access to a running wheel. The ANOVAs on these data are presented in the legend to Fig. 6. Early-morning baseline levels of plasma corticosterone, at 0800 h,

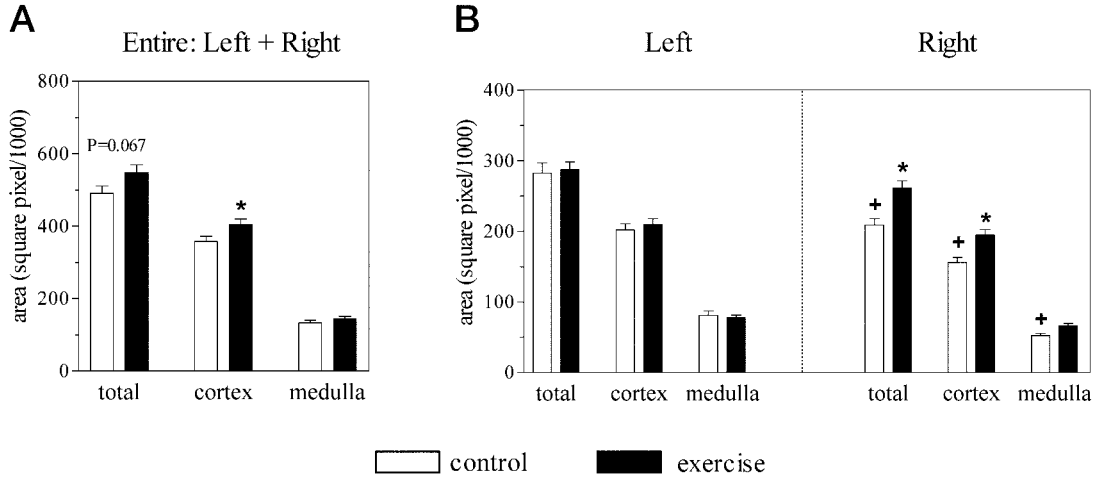

FIG. 4. Changes in the size of the left and right adrenal gland in exercising (4 wk) and control mice. Quantitative measurements of areas, by computerized image analysis of either the entire (*i.e.* left + right) adrenal surface (A) or the left and the right adrenal gland separately (B). In both A and B, the total surface and the medullar surface were measured and, by subtraction, the cortical surface was determined. Data are presented as means ± SEM (n = 7). For more details, see *Materials and Methods* and *Results*. *, Significant difference between exercise and control (*post hoc* tests with contrasts); +, significant difference between left *vs.* right within "total," "cortex," or "medulla" and within the same treatment group (*post hoc* tests with contrasts).

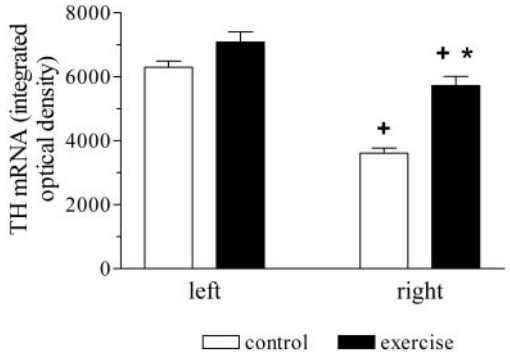

FIG. 5. Changes in TH mRNA levels in the adrenal medulla of control and (4-wk) exercising mice (n = 10 for both groups). TH mRNA was detected by *in situ* hybridization histochemistry, and autoradiograms were analyzed by computerized image analysis. TH mRNA levels are expressed as integrated optical density (*i.e.* nett gray values × square pixels/1000). For more details, see *Materials and Methods* and *Results*. Data are presented as means ± SEM. *, Significant difference between exercise and control within the left or right adrenal medulla (*post hoc* tests with contrasts); $^{+}$, significant difference between left and right adrenal medulla within the same treatment group (*post hoc* tests with contrasts).

were not different between groups, albeit plasma ACTH concentrations at this time were significantly lower in exercising mice than in control animals (Fig. 6, A and B). By the end of the light period (*i.e.* 1800 h), plasma corticosterone levels were twice as high in exercising mice than in control animals, whereas ACTH levels were comparable. Plasma hormone levels at midnight were not different between groups.

To investigate whether the changes in plasma corticosterone secretion might be of biological significance, we investigated the plasma concentration of CBG, which is the primary transport protein of corticosterone in rodents. Although significantly higher CBG levels were found in running-wheel mice at 1800 h (Fig. 6C), the extent of the elevation was much less than that in corticosterone levels (Fig. 6B). Therefore, an enhanced glucocorticoid biological action in the exercising mice may be expected toward the onset of the dark, behaviorally active period.

To assess whether long-term exercise affects the HPA axis response to stress, control and exercising mice were challenged with various stressors. Figure 7 shows that, although plasma ACTH levels were similar in the experimental groups after either 10 min of forced swimming (Fig. 7A) or 30 min of restraint (Fig. 7C), the response in plasma corticosterone was significantly higher in the exercising mice (Fig. 7, B and D). A similarly differentiated response in plasma ACTH and corticosterone levels in control *vs.* exercising mice was observed after 15 min of social defeat (data not shown).

To explore the impact of a mild psychological stressor, control and exercising mice were challenged with novelty by placing them in a new cage. The exercising mice, however, were used to having a running wheel in their cage and, thus, a new cage without a running wheel might have a relatively higher impact in these mice than in control animals. This because the control mice were not used to having a running wheel anyway. Therefore, in some cases, we varied the novel environment condition by placing a clean running wheel in

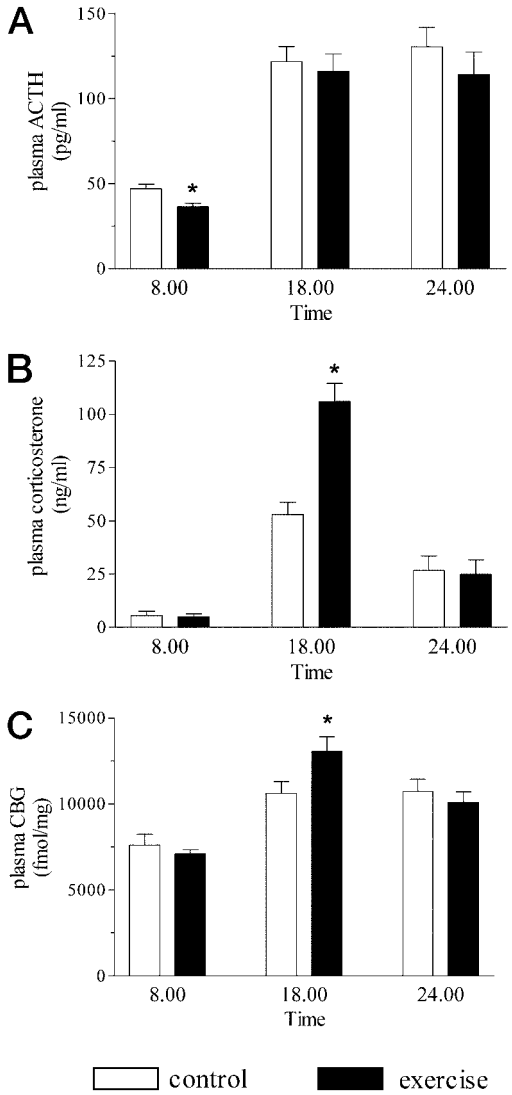

FIG. 6. Circadian rhythm of plasma ACTH, corticosterone, and CBG levels (presented as means ± SEM) in control and (4-wk) exercising mice. Animals were killed under baseline conditions either at 0800 h (n = 14–15; except for CBG, n = 8), at 1800 h (n = 8), or at 2400 h (n = 8). Exercising mice showed lower morning ACTH levels, whereas plasma corticosterone and CBG levels at 1800 h were increased. ANOVA: ACTH, effect of time, $F(2, 55) = 88.2$, $P < 0.0001$; effect of exercise, $F(1, 55) = 3.1$, $P > 0.05$; interaction time × exercise, $F(2, 55) = 0.2$, $P > 0.05$. Corticosterone: effect of time, $F(2, 55) = 127.1$, $P < 0.0001$; effect of exercise, $F(1, 55) = 17.9$, $P < 0.05$; interaction time × exercise, $F(2, 55) = 19.1$, $P < 0.0001$. CBG: effect of time, $F(2, 42) = 24.8$, $P < 0.0001$; effect of exercise, $F(1, 42) = 0.7$, $P > 0.05$; interaction time × exercise, $F(2, 42) = 3.7$, $P < 0.05$. *, Significantly different from control mice at the respective time point (*post hoc* tests with contrasts).

the new cage. Figure 8A shows that, overall, the plasma ACTH response to novelty was lower in exercising mice [effect of exercise, $F(1, 43) = 10.466$, $P < 0.005$]. The presence of a running wheel in the new cage did not influence the ACTH responses of either the control mice or the exercising animals.

The plasma corticosterone data (Fig. 8B) presented a distinctly different response pattern than the ACTH data. Regarding plasma corticosterone, the size of the response de-

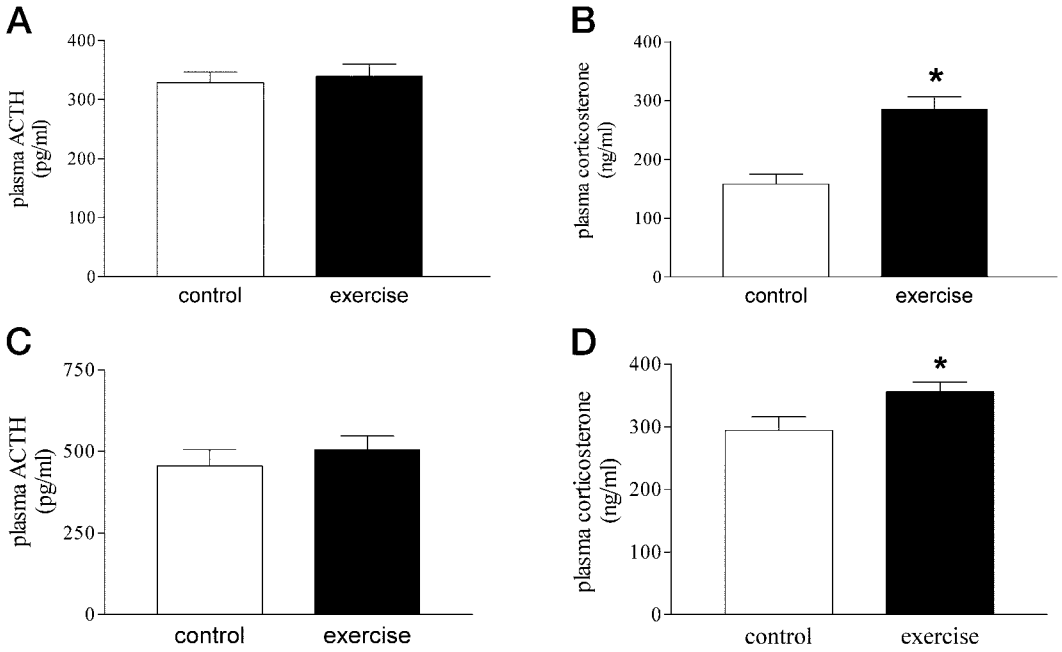

FIG. 7. Plasma ACTH and corticosterone levels in control and (4-wk) exercised mice killed either immediately after 10 min of forced swimming (A and B, respectively) or after 30 min of restraint stress (C and D, respectively). Regarding both stressors, exercising mice showed rises in plasma ACTH levels similar to those of the control animals, but the rises in plasma corticosterone levels were significantly higher than those in the controls. *, $P < 0.05$, Student's t test, means ± SEM, n = 8 for all groups.

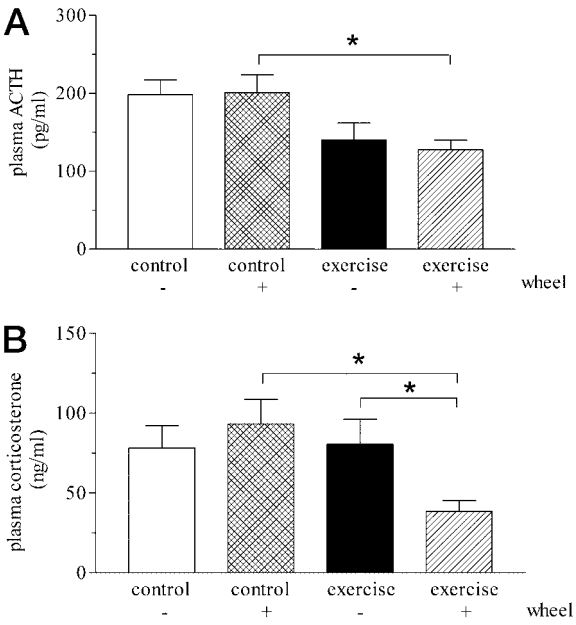

FIG. 8. Plasma ACTH and corticosterone levels in control and (4-wk) exercised mice killed after 30-min novel environment stress (*i.e.* new cage). For some control and exercising animals, the new cage was furnished with a clean running wheel. For details, see *Materials and Methods* and *Results*. Data are expressed as means ± SEM (n = 9–12). *, Significant difference (*post hoc* tests with contrasts).

pended on the presence of a running wheel in the novel environment [interaction between "exercise" and "running wheel in novel cage," $F(1, 44) = 4.304$, $P < 0.05$], this is in contrast to the plasma ACTH responses. Figure 8B shows that exercising mice showed a reduced corticosterone response only when a running wheel was included in the novel environment. Moreover, in exercising mice, omission of a running wheel in the novel environment resulted in significantly higher corticosterone responses despite similar ACTH responses. This is also reflected by the substantially lower ACTH/corticosterone ratios in novelty-challenged exercising mice minus running wheel *vs.* those in exercising animals plus running wheel [*i.e.* 2.2 ± 0.5 (n = 9) *vs.* 3.7 ± 0.4 (n = 9), Student's t test, $P < 0.05$].

We noted that exercised mice, when given a running wheel in the new cage, indeed ran in it for a significant part of the 30-min period (whereas the control mice did not). To test whether the ability to actually run in the wheel or the simple physical presence of a wheel would be critical for the HPA response in the new cage, exercised mice were offered a malfunctioning running wheel (*i.e.* with a blocked turning mechanism). The HPA hormone responses in these animals were similar to those found in the control mice [*i.e.* plasma ACTH, 310 ± 28 pg/ml (n = 7); plasma corticosterone, 105 ± 12 ng/ml (n = 7)], thus significantly higher than the levels found in the exercising mice provided with a functional running wheel. These data suggest that a functional running wheel seems to be critical to restrain the plasma corticosterone in the exercising mice.

Neuropeptide mRNA expression in the hypothalamic PVN

To explore whether changes in neuropeptide expression in the hypothalamic PVN may be involved in the altered HPA responses observed in the running-wheel mice, we conducted *in situ* hybridization histochemistry for CRF, vasopressin, and oxytocin mRNA on sections of control and exercising mice. We observed a significant decrease in CRF mRNA expression in the PVN of exercising mice, whereas no

TABLE 1. Changes in neuropeptide expression in the hypothalamic PVN after long-term voluntary exercise

mRNA	Control	Exercise
CRF	89 ± 2	79 ± 3[a]
Vasopressin	124 ± 9	124 ± 4
Oxytocin	137 ± 10	141 ± 10

CRF, vasopressin, and oxytocin mRNA levels in the PVN of exercising and control mice. Exercising animals had access to a running wheel for a 4-wk period. mRNA levels were determined by *in situ* hybridization histochemistry and semiquantitative measurement of grey values by computerized image analysis (for details, see *Materials and Methods*). Data are expressed as nett grey values (mean ± SEM, n = 6–8).

[a] $P < 0.05$, Student's t test.

changes were observed in both vasopressin and oxytocin mRNA levels (Table 1).

Corticosteroid receptor binding in brain and pituitary

To determine whether changes in glucocorticoid-binding MR and GR levels might underlie the HPA changes observed in exercising mice, the binding levels of these receptors were measured in the hippocampus. In the hippocampus of exercising mice, a significant reduction in MR levels (−38%, $P < 0.05$, Student's t test) was observed, whereas the reduction in GR levels did not reach statistical significance ($P > 0.05$; Fig. 9).

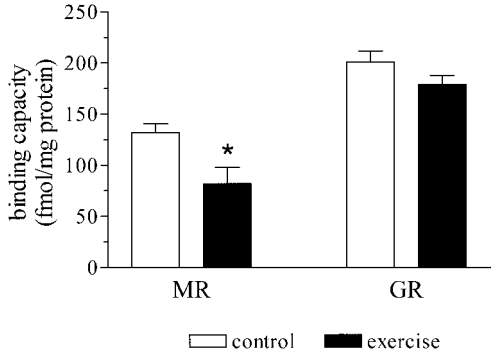

FIG. 9. Corticosteroid receptor binding capacity in mouse hippocampus tissues after 4 wk of voluntary exercise. Mice were killed 1 d after adrenalectomy. MR and GR binding was determined by radioligand binding assay (for details, see *Materials and Methods*). Receptor levels were determined in tissues of individual animals at a single 95%-saturating radioligand concentration in triplicate (data presented as means ± SEM, n = 9 animals per group). For more details, see *Materials and Methods*. *, $P < 0.05$, Students t test.

Discussion

In the present study, we showed that 4 wk of voluntary exercise leads to marked changes in HPA axis regulation and body composition in mice. At baseline, exercising mice showed lower early-morning plasma ACTH levels and substantially higher plasma corticosterone levels at lights-off. These mice also presented heavier adrenal glands, which, however, was almost selectively attributable to an enlarged right adrenal cortex. Strikingly, the adrenal medulla of exercising mice expressed higher levels of TH mRNA, suggesting, at least episodically, increases in sympathoadrenomedullary activity. After stress, stressor-specific changes in HPA hormone responses were observed. Overall, stressors with a strong physical component, such as forced swimming, induced an exaggerated corticosterone response in exercising mice, whereas a mild psychological challenge (*i.e.* a novel environment) resulted in attenuated hormone responses. Our data suggest that long-term voluntary exercise evolves in complex changes at different levels of the HPA axis, resulting in varying hormonal responses to physical *vs.* psychological challenges.

The mice in our study ran approximately 4 km per day, which they performed almost completely during the nighttime. This observation is in agreement with other reports (26a, 27, 28). Mice run voluntarily when offered a running wheel. Thus, it seems that it complies with a natural urge of the animals (39, 40), increasing physical fitness (41) and helping to control body weight (41, 42). Wheel running is not regarded as a form of stereotypic behavior (27) because it is not expressed at the cost of resting behavior, as is the case with other reported locomotor stereotypes (43, 44).

No changes were observed in body weight, which is in agreement with reports from others (27, 45). However, body composition is changed in exercising mice, mainly because of substantial loss of abdominal adipose tissue (Refs. 42 and 46 and present study), skeletal muscle enlargements (47), and increases in heart weight (Refs. 42 and 47 and references therein). The reduction in adipose tissue is a result of an enhanced lipolysis in these animals (46). Food intake in the exercising mice was unchanged, corresponding with previous observations (27). The increased water consumption may be the result of an enhanced evaporation because of increases in body temperature and breathing frequency to be expected during exercise. Thus, voluntary exercise results in marked physical and physiological changes in mice.

Our exercising mice presented increased adrenal weights and decreased thymus weights, which is consistent with the risen glucocorticoid levels during a part of the diurnal cycle, *i.e.* during the first half of the dark phase. Increased adrenal weights after exercise is a long-known finding (48–50). Apart from these changes in the weight of the adrenals, we revealed left *vs.* right differences and exercise-induced changes in the adrenal glands. In control mice, the left adrenal gland was substantially larger than the right one, a phenomenon that has been observed also in other species (51, 52). Here, we show that this is attributable to both a larger cortex (+29%) and larger medulla (+54%). This asymmetry was not restricted to C57BL/6N mice but was also observed in other strains (*e.g.* B6C3F1 mice; Droste, S. K., S. Ulbricht, and J. M. H. M. Reul, unpublished observation). It indicates furthermore that sympathoadrenomedullary activity may be asymmetric, with a higher activity on the left side. This finding corresponds with the concept of a predominant involvement of the right brain hemisphere in the control of sympathetic activity and the higher levels of noradrenaline found in this side of the brain (53, 54). It should be noted, however, that asymmetry in the autonomic nervous system has been only scarcely studied so far. Nevertheless, our finding on the left-right difference in adrenomedullar size strengthens the concept on sympathetic asymmetry.

In exercising mice, the asymmetry in the size of the adrenal glands, seen in the control animals, was abolished. This was

mainly attributable to a significant increase in the size of the right adrenal cortex (+25%), thereby becoming as large as its left counterpart. Although the enlarged right adrenal cortex in exercising mice corresponds with the enhanced glucocorticoid secretion in these animals at the onset of the dark phase and with their enhanced energy requirement, the question remains as to why this only concerns the right adrenal cortex. In this respect, a key observation may be the asymmetric increase in TH mRNA levels in the adrenal medullas after exercise. We observed a significant rise in TH mRNA levels only in the right medulla, suggesting a higher impact of sympathoadrenomedullary input in the right medulla than in the left one. Interestingly, more than two decades ago, it was already shown that neural inputs to the adrenal glands are important growth determining factors for the adrenal cortices (55–57). Thus, the selective enlargement of the right adrenal cortex in the exercising mice may be attributable to an intensified right-sided sympathoadrenomedullary input. Yet, it remains to be clarified in future studies why mainly the right branch of the sympathoadrenomedullary input is intensified after long-term voluntary exercise and not both branches. Furthermore, the role of ACTH herein needs to be addressed as well as the question of whether adrenal afferent fibers participate in this neural mechanism (57, 58). Summing up, exercising mice present a loss of adrenal asymmetry caused by adrenocortical enlargement and intensified sympathoadrenomedullary input in the right gland.

In contrast to our observations in voluntarily exercising mice, other researchers observed substantially enlarged adrenal medullas in animals and man after exercise training (59–61). However, in those cases, subjects were subjected to either high-intensity exhaustive exercise [in man (59)] or forced to run in a treadmill usually on a cumulative performance schedule [in rats (60–62)]. These observations further underscore that our voluntary exercise paradigm is not comparable with those models and that exhaustive exercise induces changes that go beyond the ones seen after voluntary exercise.

In our discussions, until now, we have paid considerable attention to the exercise-induced changes in the adrenal glands. The main reason for this is that changes in the sympathoadrenomedullary system could be pertinent for explaining the changes observed in the hormonal secretion patterns of the HPA axis. This may already come into play in the doubled plasma corticosterone levels, in the face of unchanged ACTH levels at the onset of the dark phase. The augmented adrenocortical responsiveness to ACTH is possibly caused by the enhanced sympathoadrenomedullary activity at this time (59, 63), because mice run mainly during the first half of the dark phase (26a). This fits also with the normal corticosterone levels at midnight. As mentioned, sympathetic activity in the adrenal medulla is known to be a positive modulator of adrenocortical sensitivity to ACTH (38). The corticosterone surge at 1800 h presumably can be regarded as anticipatory and adaptive to support metabolism for the upcoming physical activity. However, the observation that no significant GR down-regulation was found in hippocampus tissues and that the thymus involution in exercising mice was only moderate underscores the fact that apparently the glucocorticoid hypersecretion in these mice was of limited magnitude and duration. Also, the elevated circulating levels of CBG contribute to restrain the biological impact of the glucocorticoid hypersecretion. The decrease in hippocampal MR density of exercising mice was unexpected, because these receptors are rather resistant to homologous down-regulation, this in contrast to brain GRs (64). Nevertheless, a number of studies have shown that, at least in the rat, MR levels can be (homologously) down-regulated by glucocorticoid hormones (mostly corticosterone) (65–68). Therefore, it cannot be excluded that the recurrent phases of elevated circulating glucocorticoid levels in the exercising mice might be the cause of the down-regulation of hippocampal MR levels. That the situation regarding glucocorticoid regulation of MR is not really clear yet, however, is indicated by the observations that both the synthetic glucocorticoid agonist dexamethasone and the synthetic glucocorticoid antagonists RU 38486 and ORG 34517 up-regulate hippocampal MR levels (25, 64, 69, 70). The brain MR is also known to be regulated by neuropeptides [such as CRF (33)], neurotransmitters [such as serotonin (71) and noradrenaline (72, 73)], and growth factors (74). However, serotonin and β-adrenoceptor agonists can be ruled out, because they are known to be positive regulators of MR. The decrease in hippocampal MR could be evoked by α-adrenergic stimulation, given that: α-adrenoceptor agonists and stress-induced increases in (nor)adrenaline suppress MR mRNA levels (73); brain noradrenaline levels are increased during exercise and spontaneous behavioral activity (75–77); and α-adrenergic receptors and MRs are coexpressed in hippocampal pyramidal neurons (78). Whether other factors and/or other mechanisms (*e.g.* desensitization of signal transduction pathways) play a role in the MR decrease awaits further investigation.

We observed decreased early-morning resting plasma ACTH levels in the exercising mice, in which changes in both driving and negative feedback mechanisms may be playing a role. Although we found decreased CRF mRNA levels in these mice, it is unclear whether they may be indicative for a decreased drive in the hypothalamic-pituitary axis, given that CRF has been shown to be mainly involved in stress-induced ACTH responses and not in maintaining early-morning baseline ACTH levels (79, 80). Alternatively, the reduced ACTH levels may result from enhanced negative feedback influences via GRs attributable to the elevated glucocorticoid levels during the first half of the dark phase. The decreased hippocampal MR levels can hardly be held responsible for the decreased ACTH levels, because, given the known MR-mediated tonic inhibition of PVN parvicellular neurons (25, 33, 81–83), rather elevated ACTH levels were to be expected. Thus, it seems that, in exercising mice as a result of the enhanced glucocorticoid action via GRs, a compensatory decline in MR density has taken place, most likely to maintain the homeostatic balance between negative feedback and responsiveness within the HPA axis (see also Ref. 24). Previously, we have shown that the density of MRs in hippocampus and other limbic regions can indeed change rapidly in response to varying needs (33). The sympathetic nervous system participates in this network as it modulates adrenocortical responsiveness to ACTH via its sympathoadrenomedullary output and, interestingly, it is modulated itself by glucocorticoids via MRs and GRs (84, 85).

Regarding the effects of stress, we observed stressor-specific differential responses in plasma ACTH and corticosterone in exercising *vs.* control mice. The animals produced similar ACTH responses to forced swimming and restraint, but the exercising mice mounted substantially higher corticosterone responses to these stressors. The ACTH data show that, despite lower paraventricular CRF mRNA levels, exercising mice apparently can produce a normal ACTH response to such potentially life-threatening situations. Thus, their hypothalamic-pituitary axis is not functionally incapacitated. Moreover, also the negative feedback efficacy seems to be normal in exercising mice [this in contrast to observations in some endurance-trained men (23)], given the normal peak responses in ACTH and the unchanged GR capacity in these animals. The higher glucocorticoid response in exercising mice to forced swimming and restraint corresponds with the enlarged adrenal cortex (present study), the elevated TH mRNA expression (present study), and the reportedly enhanced sympathoadrenomedullary activation in response to stressors demanding physical activity (59, 86, 87) and to psychosocial stress (88). Although unlikely, it cannot be completely excluded that differences in plasma ACTH, after these stressors, were missed, which could account for the differences in plasma corticosterone, because only one post-stress sample was collected. Other researchers found, in forced exercised rats, blunted plasma ACTH and unchanged corticosterone responses to foot shock and forced swimming (89–91). These observations underline, once more, that forced exercise induces different regulatory changes in the HPA axis than voluntary exercise and can be regarded as a chronic stress paradigm.

Thus, our exercised mice respond to stressors comprising a strong physical component with augmented glucocorticoid responses, presumably because their HPA and sympathoadrenomedullary axis have adapted to meet the enhanced metabolic demand during running. However, they react in a novel environment with different HPA hormone responses. Regardless of the presence of a running wheel in the novel environment, the plasma ACTH responses in the exercised mice were markedly lower than those in the sedentary mice, clearly indicating that the exposure to novelty has lower impact in these animals. This observation is consistent with studies reporting that exercised mice (Binder, E., S. K. Droste, F. Ohl, and J. M. H. M. Reul, unpublished data) and humans (13–15) show reduced anxiety. The plasma corticosterone response in our exercising mice depended critically on the presence of a functional running wheel in the new cage. In the absence of the running wheel, the exercising animals produced the same glucocorticoid levels as the control mice, which can be explained by the adrenocortical hyperresponsiveness we have generally observed in the exercising mice. However, if the exercising animals had access to a running wheel in the new cage, then the corticosterone responses were much lower. The animals indeed used the running wheel for a significant amount of the exposure time. Thus, the use of the running wheel may be regarded as displacement behavior, resulting in a further reduction of the emotional impact of the novel environment. A similar effect on corticosterone secretion has been observed when rats were given access to a running wheel after being exposed to a foot shock paradigm (92). The exact mechanism by which the glucocorticoid response is restrained under these conditions is presently unknown, but it may involve reduced sympathoadrenomedullary outflow and local adrenomedullary inhibitory mechanisms.

Exercise training has been shown to exert anxiolytic and antidepressant effects in depressed patients and to increase mood in normal subjects (13–15, 93). The exact neurobiological mechanism underlying these effects of exercise is still unclear, but parallels with the mechanism of action of antidepressant drugs exist. Both exercise and antidepressants bring patients relief of their anxiety and depressed mood, improved stress coping, and increased vegetative stability (13–15). Most depressed patients show major disturbances in their regulation of the HPA axis, and evidence is accumulating that a normalization of this neuroendocrine system is a prerequisite for stabile remission of the depressive symptomatology (for review, see Refs. 25, 94, and 95). Animal experiments have shown that long-term antidepressant treatment results in reduced HPA hormone responses to a mild psychological stressor (*i.e.* novel environment) but not to more severe stressors such as forced swimming (25, 96) (Gesing, A., and J. M. H. M. Reul, unpublished data). Clearly, there is a striking parallel between these antidepressant effects and the here-presented effects of voluntary exercise on stress-induced HPA hormone responses. Furthermore, both antidepressant treatment (97) and voluntary exercise (present study) reduce PVN CRF mRNA levels. In view of these parallel effects, which are also evident in the effects on brain plasticity processes [*e.g.* neurogenesis (9, 10, 98)], it is tempting to speculate that the antidepressant and anxiolytic effects of exercise and antidepressant drugs pertain overlapping neurobiological mechanisms involving a dampening of HPA responses to emotional stressors. Therefore, future research on the effects of exercise on the brain may reveal novel targets for antidepressant drugs or even lead to the discovery of endogenous antidepressant and/or anxiolytic molecules.

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