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ORIGINAL ARTICLE

Long-Term Voluntary Exercise and the Mouse Hypothalamic–Pituitary–Adrenocortical Axis: Impact of Concurrent Treatment with the Antidepressant Drug Tianeptine

S. K. Droste,*† M. C. Schweizer,* S. Ulbricht* and J. M. H. M. Reul*†

**Max Planck Institute of Psychiatry, Section of Neuropsychopharmacology, Munich, Germany.*

†Henry Wellcome Laboratories of Integrative Neuroscience and Endocrinology, Dorothy Hodgkin Building, University of Bristol, Bristol, UK

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We investigated whether voluntary exercise and concurrent antidepressant treatment (tianeptine; 20 mg/kg/day; 4 weeks) exert synergistic effects on the mouse hypothalamic-pituitary-adrenocortical (HPA) axis. Animals had access to a running wheel, were treated with the antidepressant, or received both conditions combined. Control mice received no running wheel and no drug treatment. Exercise resulted in asymmetric changes in the adrenal glands. Whereas sedentary mice had larger left adrenals than right ones, this situation was abolished in exercising animals, mainly due to enlargement of the right adrenal cortex. However, antidepressant treatment alone was ineffective whereas the combination of antidepressant treatment and exercise resulted in an enlargement of both adrenal cortices. In these respective conditions, the levels of tyrosine hydroxylase (TH) mRNA expression in the left and right adrenal medullas varied greatly in parallel to the changes observed in the adrenal cortex sizes. TH mRNA expression in the locus coeruleus of exercising mice was significantly increased irrespective of concomitant tianeptine treatment. Corticotrophin-releasing factor mRNA levels in the hypothalamic paraventricular nucleus were decreased after voluntary exercise but were unaffected by tianeptine. Exercise, particularly in combination with tianeptine treatment, resulted in decreased early morning baseline plasma levels of corticosterone. If animals were exposed to novelty (i.e. a mild psychological stressor), a decreased response in plasma corticosterone levels was observed in the exercising mice. By contrast, after restraint, a mixed physical and psychological stressor, exercising mice showed an enhanced response in plasma corticosterone compared to the controls; a response which was even further boosted in exercising mice concomitantly treated with tianeptine. Under either condition, plasma adrenocorticotrophic hormone levels were not different between groups. Thus, voluntary exercise impacts substantially on HPA axis regulation. Concurrent tianeptine treatment results in synergistic actions, mainly at the adrenal level, affecting both its structure and function.

Key words: voluntary exercise, HPA axis, tianeptine, antidepressant, corticotrophin-releasing factor, mice, tyrosine hydroxylase.

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Correspondence to:
Professor J. M. H. M. Reul, Henry Wellcome Laboratories of Integrative Neuroscience and Endocrinology, Dorothy Hodgkin Building, University of Bristol, Whitson Street, Bristol BS1 3NY, UK (e-mail: hans.reul@bristol.ac.uk).

The regular performance of exercise has vast beneficial effects on a variety of biological systems. Most research has been carried out with respect to weight control (1, 2) and the cardiovascular system (3, 4). However, evidence is accumulating that regular exercise also impacts positively on the brain, resulting in antidepressant-like and anxiolytic effects (5, 6).

At the level of the brain, it has been shown that several processes are positively affected by voluntary exercise. The observation attracting the greatest interest in recent years is that exercise leads to increased neurogenesis in the dentate gyrus of the hippocampus, which is thought to be the result of an enhanced action of growth factors [e.g. insulin growth factor-1, brain-derived neurotrophic

factor (BDNF)] in the brain (7, 8). An increased neurogenesis in the dentate gyrus is also seen after antidepressant treatment (7, 9). The action of voluntary exercise and antidepressant drugs such as tianeptine on neurogenesis and other neuroplasticity processes may involve changes in glutamatergic neurotransmission (9–11).

Recently, we reported that long-term voluntary exercise (by allowing access to a running wheel) leads to significant changes at different levels of the hypothalamic-pituitary-adrenal (HPA) axis in mice (12, 13). These changes included decreased HPA hormone responses to emotional stimuli such as exposure to a novel environment. Moreover, in a recent behavioural study, we showed that exercising mice are able to cope better with emotional stimuli. We observed that long-term voluntary exercise significantly decreases anxiety-related behaviour and impulsivity (6). Antidepressant treatment has been shown in rodents to elicit comparable stress-related effects on the HPA axis and behaviour: The HPA axis activity is decreased in response to stressors and the animals show less anxiety-related behaviour (14, 15). Moreover, Lancel *et al.* (16) found that exercising mice show increased sleep consolidation and reduced rapid-eye-movement (REM) sleep, suggesting improved sleep quality; this is also a known effect of antidepressant drugs in depressed patients (17).

Studies in healthy subjects and patients have revealed behavioural and neuropsychological changes associated with regular physical exercise. It has been shown that exercise training increases mood in normal subjects and evokes anxiolytic and antidepressant effects in phobic and depressed patients (5, 18). It is well known that depressed patients often show a disturbed HPA axis compared to healthy subjects, and that the clinical recovery after antidepressant treatment is associated with a normalisation of HPA axis function (19).

Besides overlapping effects of antidepressant drug action and physical activity, there also appear to be synergistic effects as shown, for example, on BDNF expression in the rat hippocampus (7, 20). Furthermore, there is evidence to suggest that exercise has beneficial effects on the clinical course of antidepressant-treated patients suffering from depression (21). Therefore, in view of the important role of the HPA axis in major depressive disorder, we aimed to investigate whether exercise and antidepressant cotreatment would exert synergistic effects on this neuroendocrine system.

Materials and methods

Animals

Male C57BL/6N mice (age on arrival, 10–12 weeks; Charles River, Sulzfeld, Germany) were singly housed in Macrolon type III cages (43 cm × 24 cm × 15 cm) under a 12 : 12 h light/dark cycle (lights on 06.00 h) at 22–23 °C and 50–60% relative humidity. Food and water were available *ad libitum*. All animal experiments were approved by the government of Bavaria, Germany.

Antidepressant drug treatment

The antidepressant drug tianeptine (Servier, France) was used in our studies. It is an antidepressant with a structure similar to tricyclic antidepressants (TCAs) but with a different pharmacological profile. Tianeptine has been shown to regulate neuroplasticity (11) and to stimulate the uptake of serotonin but not of dopamine and noradrenaline, by cortical and hippocampal rat synaptosomes *in vitro* (11, 22, 23). Tianeptine has the same clinical efficacy regarding the treatment of major depression as TCAs and selective serotonin reuptake inhibitors (24, 25). In the present study, treatment of the mice was at a dosage of 20 mg/kg body weight per day. To prevent nonspecific stress effects of repeated injections, animals received the antidepressant via their drinking water. Because tianeptine is light sensitive, the drug solution was given in dark bottles and renewed every day. Liquid intake over a 24-h period was determined at least twice each week. Data were averaged to produce a mean liquid intake for each week and mouse (ml/day). The drug concentration in the drinking water was carefully adjusted throughout the treatment to compensate for the increase in the body weight and changes in liquid intake over the experimental time period. This was performed to ensure that a dosage of 20 mg/kg/day was indeed maintained for each drug-treated animal over the whole experimental time period. This protocol was particularly important in view of the fact that the exercising drug-treated mice drank significantly more than the sedentary drug-treated animals. Thus, both drug-treated groups received the same dosage of antidepressant over the complete period of treatment. Finally, the control mice received tap water.

Voluntary exercise paradigm

After habituation to the housing conditions for 5 days, the experimental groups 'exercise' and 'exercise + antidepressant' were allowed free access to a running wheel (14 cm in diameter) in their home cages for 4 weeks. At this time also the antidepressant treatment commenced. 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 first half of the dark phase of the diurnal cycle (16). Importantly, wheel running is not regarded as a form of stereotypic behaviour (26) because it is not expressed at the cost of resting behaviour, as is the case with the reported locomotor stereotypies (27, 28). The housing conditions of the sedentary (i.e. 'control') and 'antidepressant' animals remained unchanged.

Assessment of physical measures

The weight of the animals was determined weekly. At the time of death (i.e. after 4 weeks of exercise), antidepressant treatment or control conditions, brain, adipose, thymus and adrenal tissues were collected. Only tissues of mice killed under baseline conditions (i.e. nonstress conditions) were collected for physical and gene expression measures.

The abdominal (i.e. peritoneal + perirenal) adipose tissue and thymus were weighed. 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 cryostat, and mounted on slides (Superfrost, Menzel-Gläser, Merck Eurolab GmbH, Ismaning, Germany) previously coated with poly L-lysine (Sigma, Deisenhofen, Germany). The adrenal sections were stored at −20 °C until staining. Staining and measurement of adrenal medullar and cortical areas were conducted exactly as described before (12). 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 added to obtain one value for the two adrenals together for total, cortical and medullar area per animal. Subsequently, mean ± SEM values were calculated for each experimental group.

Assessment of baseline and stress-induced HPA axis activity

After the experimental period, the animals were killed either under early morning baseline conditions (07.00–09.00 h) or exposed to a novel environ-

ment or a restraint stress procedure. For the novel environment exposure, mice were placed singly in clean cages containing new sawdust and no food and water for 30 min, after which they were quickly killed, and trunk blood was collected as outlined below. As an extra variable, half of the control and exercising mice were provided additionally with a clean running wheel in the new cage. This extra variable was introduced to check for the differential impact that the novel environment might have on the different groups of mice. This in view of the fact that the exercising mice were used to having a running wheel in their home cage whereas the sedentary animals were not used to this condition. Restraint stress was achieved by putting the animals in a clear plastic 50-ml Falcon-like tube (Greiner, Frickenhausen, Germany), with a diameter of 3 cm and containing ventilation holes, for 30 min. After completion of the restraining procedure, the mice were quickly killed and trunk blood was collected as described below.

As reported previously (12), for killing, individual mice were quickly anaesthetised (< 15 s) in a glass jar containing saturated isoflurane (Curamed, Karlsruhe, Germany) vapour, after which the animals were decapitated immediately. This rapid procedure of anaesthesia before killing does not affect baseline HPA hormone levels (Droste and Reul, unpublished observations). Trunk blood was collected in ice-chilled EDTA-coated tubes (1.5 ml) containing 25 μg aprotinin (Trasylol, Bayer, Germany) for preparation of plasma and hormone measurements.

Hormone measurements

Plasma samples for adrenocorticotrophic hormone (ACTH) and corticosterone measurement by radioimmunoassay (ICN Biomedicals, Costa Mesa, CA, USA) were stored at −80 °C and −20 °C, respectively. The inter- and intra-assay coefficients of variance for ACTH were 7% and 5%, respectively, with a detection limit of 2 pg/ml. For corticosterone, the inter- and intra-assay coefficients of variance were 7% and 4%, respectively, with a detection limit of 0.4 ng/ml.

In situ hybridisation 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 cryostat 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, Karlsruhe, Germany). In addition, adrenal glands were collected, frozen on dry ice, and cut into 12-μm thick sections in a cryostat.

In situ hybridisation for vasopressin (AVP), oxytocin and tyrosine hydroxylase (TH) mRNA were performed using oligodeoxynucleotide probes whereas detection of corticotrophin-releasing factor (CRF) mRNA was performed using a ^{35}S-labelled antisense cRNA probe. An elaborate description of the method has been previously provided (12). Radioactivity in the dried sections was detected by exposure to autoradiography film (Kodak Biomax MR-1, Kodak, NY, USA) for 2–4 days.

Optical densitometry

Representative autoradiograph images of the hypothalamic PVN or the locus coeruleus (LC) were digitally recorded using a CCD video camera (XC-77CE, Sony, Tokyo, Japan). Semi-quantitative analyses of mRNA expression were performed blind using a densitometric video image analysis system (Optimas 5.2, Media Cybernetics, Bothell, WA, USA). The optical density (grey values, expressed as arbitrary units; resolution: 256 levels) of an area encompassing the PVN or the LC was determined and the background signal (measured just outside the PVN or LC in an area containing no apparent hybridisation signal) was subtracted. From each animal, at least three sections of a representative assay were analysed. Each assay was repeated at least three times. The optical density data of one representative assay are presented as net grey values (mean ± SEM of six to eight mice per 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* hybridisation assays. Three assays were conducted on adrenal sections of ten control and ten exercising mice. In each assay, three sections per adrenal gland were used. Autoradiograms of all sections were densitometrically analysed providing a mean value per adrenal medulla per animal. Here, data are presented of one representative assay. The other two assays provided similar data.

Semi-quantitative analysis of the TH mRNA expression level in the adrenal medulla was performed in a similar way as described for the analysis of mRNA expression in the PVN and LC. The optical density (grey 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, since 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 = net grey value × square pixels/1000.

Statistical analysis

The data on running performance and liquid intake were tested for statistically significant differences by two-way ANOVA with repeated measures followed in appropriate cases by post-hoc tests with contrasts. In the case of running performance, data were averaged over time bins of 4 days (except for the last bin of 3 days) to reduce the probability of type 1 errors. The experimental data on the physical parameters, hormone levels and mRNA levels were tested with one-, two- or three-way ANOVA followed in appropriate cases by post-hoc tests with contrasts. $P < 0.05$ was considered statsically significant. 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

Wheel running performance

Figure 1 shows the running distances of mice with and without antidepressant treatment over a period of 4 weeks. Mice showed a stabile running performance from day 5 onward and ran a substantial distance per day (i.e. 6.0 ± 0.7 km/day in the case of the control mice, mean ± SEM; $n = 10$). The exercising mice treated with tianeptine presented a similar running distance up to day 5, but from then onward the average distances run increased to as much as 9.3 ± 1.0 km/day ($n = 5$) per day. There was a significant effect of time [$F(6,78) = 4.90$, $P < 0.0005$] and an effect of antidepressant treatment [$F(1,13) = 11.08$, $P < 0.01$]. Previously, we have reported that running is almost exclusively performed during the first half of the dark phase of the diurnal rhythm (16).

Liquid intake

Figure 2 shows a time course of the liquid intake over the 4-week period. The liquid intake was significantly different between the

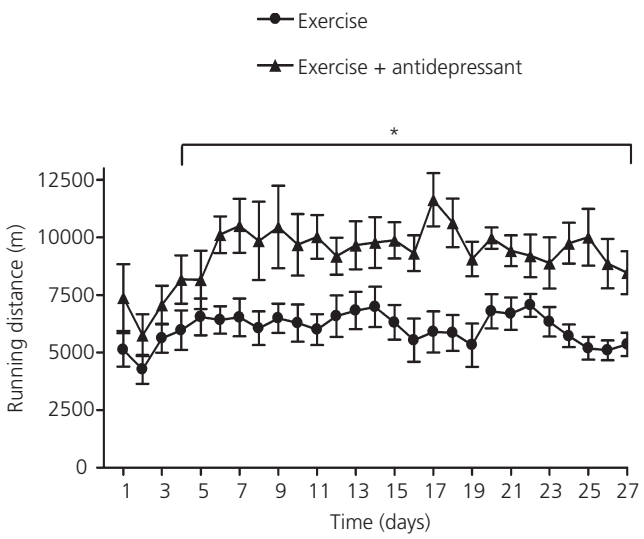

Fig. 1. Time course of daily wheel running activity (means ± SEM) of male C57BL/6N mice over a time period of 27 days. The exercising animals received either tianeptine in the drinking water (exercise + tianeptine, n = 5) or the drinking water only (exercise, n = 10). The drug concentration in the drinking water was adjusted throughout treatment to control for the increase in the bodyweight and changes in liquid intake. These adjustments were conducted throughout all studies. For ANOVA data, see text of the Results section; *P < 0.05 post-hoc tests with contrasts.

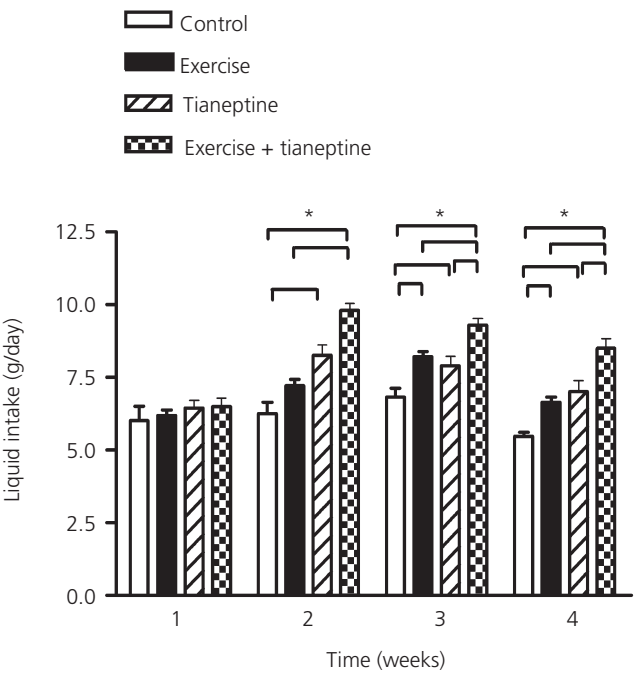

Fig. 2. Comparison of liquid intake (means ± SEM) in control, exercising and tianeptine-treated, and tianeptine-treated exercising male C57BL/6N mice (all groups: n = 10) over a time period of 4 weeks. For ANOVA data, see text of the Results section; *P < 0.05 (post-hoc tests with contrasts).

experimental groups [effect of exercise: $F(1,36) = 25.53$, $P < 0.0001$; effect of antidepressant treatment: $F(1,36) = 29.55$, $P < 0.0001$]. No interaction between exercise and the drug treatment was observed [interaction: $F(1,36) = 0.01$, not significant (NS)]. The exercise, antidepressant, and exercise + antidepressant mice showed a higher liquid intake than the control animals. This was for the antidepressant and exercise + antidepressant-treated mice significant from the second week onward, and for the exercising mice from the third week onward. Moreover, the fluid intake of the exercise + antidepressant group appeared to be the result of an additive effect of the separate conditions (Fig. 2).

Physical parameters: body and fat weight

As shown in Fig. 3(A), all four groups of mice gained weight over the 4-week time period [effect of time: $F(4,144) = 338.4$, $P < 0.0001$], but there were no differences between the groups [effect of exercise: $F(1,36) = 2.85$, NS; effect of antidepressant treatment: $F(1,36) = 0.0001$, NS; interaction: $F(1,36) = 0.7$, NS]. Figure 3(B) shows that the exercising and the exercise + antidepressant mice had significantly less peritoneal fat than the control and the antidepressant-treated animals; tianeptine alone had no effect [effect of exercise: $F(1,36) = 23.6$, $P < 0.0001$; effect of antidepressant treatment: $F(1,36) = 2.7$, NS; interaction: $F(1,36) = 0.2$, NS].

Physical parameters: thymus

The weight of the thymus did not differ significantly between the groups [control: 49 ± 2 mg (n = 10); exercise: 45 ± 2 mg (n = 10); antidepressant: 48 ± 1 mg (n = 10); exercise + antidepressant: 49 ± 1 mg (n = 10)] after the 4-week experimental period.

Physical parameters: changes in the adrenal gland

Exercise increased the size of both the cortex and the medulla (left and right added together, Fig. 4A), hence producing an overall increase in adrenal size [effect of exercise: $F(1,65) = 27.11$, $P < 0.0001$ (cortex) and $F(1,65) = 10.61$, $P < 0.05$ (medulla)]. By contrast, tianeptine was without effect [$F(1,65) = 0.0001$, NS (cortex); $F(1,65) = 0.0001$, NS (medulla)], whereas an almost significant increase in the size of the cortex was found after the combined treatment (Fig. 4A).

Separate analyses of the left and right adrenal (Fig. 4B,C) showed that the total size of the left adrenal is larger than that of the right adrenal [$F(1,65) = 42.17$, $P < 0.0001$] as a result of both a larger cortex [$F(1,65) = 32.55$, $P < 0.0001$] and medulla [$F(1,65) = 39.19$, $P < 0.0001$]. With the exception of an increase in cortex size in the left adrenal in the exercise + tianeptine-treated group, the treatments had a selective effect on the right adrenal gland (Fig. 4B,C).

TH mRNA expression in the adrenal medulla and the locus coeruleus

TH mRNA expression in the adrenal medulla was measured as an index for sympathoadrenomedullary activity (29) which is a well-known modulator of adrenocortical sensitivity to ACTH (30).

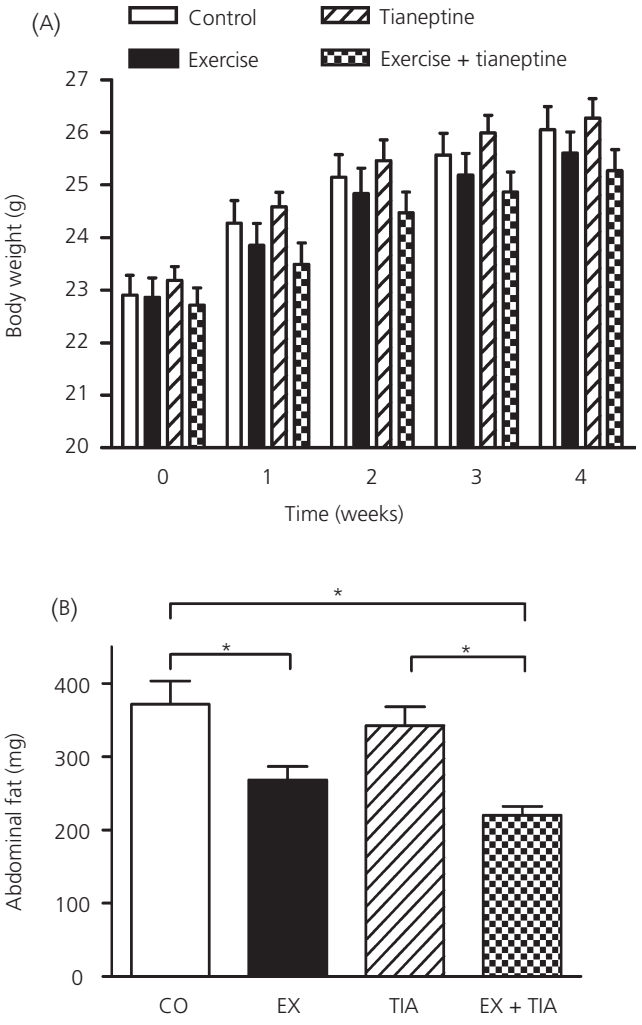

Fig. 3. Changes in (A) body weight (time course) and (B) abdominal fat tissue weight in control mice (CO; n = 10), voluntarily exercising mice (EX; n = 10), tianeptine-treated mice (TIA; n = 10) and tianeptine-treated exercising mice (EX + TIA; n = 10) over a 4-week time period. Data are expressed as means ± SEM. For ANOVA data, see text of the Results section; *P < 0.05, post-hoc tests with contrasts.

Exercise increased TH mRNA expression in the adrenal medulla (Fig. 5A) [$F(1,70) = 37.24$, $P < 0.0001$], whereas tianeptine was ineffective [$F(1,70) = 2.09$, NS, interaction exercise × tianeptine: $F(1,70) = 1.70$, NS]. In addition, TH mRNA levels were higher in the left adrenal medulla than in the right one [$F(1,70) = 39.12$, $P < 0.0001$]. Post-hoc analyses showed that exercise and the combined exercise + tianeptine treatment increased TH mRNA levels in the right medulla whereas in the left medulla an increase was only observed after the combined treatment (Fig. 5A).

In the LC, the brain region with the highest density of noradrenergic neurones, exercise increased levels of TH mRNA expression [$F(1,22) = 6.3$, $P < 0.05$] (Fig. 5B). Tianeptine had no effect [$F(1,22) = 0.2$, NS, interaction $F(1,22) = 0.0001$, NS]. Post-hoc analyses revealed no significant differences between individual experimental groups (Fig. 5B).

Baseline and stress-induced plasma ACTH and corticosterone levels

Figure 6 shows early morning baseline and stress-induced responses in plasma ACTH and corticosterone. Plasma ACTH was unaffected by either exercise or tianeptine treatment. Thus there were no significant between group differences in the plasma ACTH levels before and after novelty or restraint in mice exposed to treatments (Fig. 6A,C,E). However, regarding baseline corticosterone levels we found an overall effect of exercise [$F(1,30) = 11.224$, $P < 0.005$], no effect of tianeptine treatment [$F(1,30) = 0.038$, NS], but a significant interaction between exercise and antidepressant treatment [$F(1,30) = 4.945$, $P < 0.05$]. Baseline plasma corticosterone levels were lowest in the combined exercise plus tianeptine group (Fig. 6B).

Exercise attenuated the corticosterone response to novelty exposure [$F(1,52) = 25.82$, $P < 0.0005$], an effect which was clearest if a running wheel was present in the novel cage (Fig. 6D). Tianeptine did not affect the response to novelty. By contrast to novelty stress, the corticosterone response to restraint stress was augmented by exercise [$F(1,29) = 25.90$, $P < 0.0005$, interaction exercise × antidepressant: $F(1,29) = 3.905$, $P = 0.058$] (Fig. 6F). Moreover, although tianeptine alone was without effect, it enhanced the response to exercise (Fig. 6F).

Neuropeptide mRNA expression in the hypothalamic paraventricular nucleus

The neuropeptide expression in the hypothalamic PVN was assessed by semiquantitative *in situ* hybridisation histochemistry for CRF, vasopressin and oxytocin mRNA. We observed a significant effect of exercise on CRF mRNA expression in the PVN [effect of exercise: $F(1,16) = 13.1$, $P < 0.05$] (Fig. 7), whereas no effect of antidepressant treatment was found [effect of antidepressant treatment: $F(1,16) = 0.4$, NS; interaction: $F(1,16) = 0.5$, NS]. Post-hoc tests with contrasts showed a significant decrease in CRF mRNA levels in the exercising mice compared to the control animals, whereas levels in the antidepressant-treated exercising mice were significantly lower than those in both the antidepressant-treated mice and the control animals (Fig. 7). The mRNA levels of AVP and oxytocin were not different between the experimental groups (data not shown).

Discussion

Concurrent treatment of exercising mice with the antidepressant tianeptine produced HPA axis changes quite distinct to those evoked by the separate conditions. These differences could be observed in the effects on adrenocortical and adrenomedullar sizes, and adrenomedullar TH mRNA expression. In particular, synergistic effects of exercise and antidepressant treatment were discernable on baseline and restraint stress-induced plasma corticosterone levels in the face of an absence of effects on plasma ACTH concentrations. Our data point to an important role of the adrenal gland as a site of convergence for the synergistic actions of voluntary exercise and the antidepressant drug tianeptine.

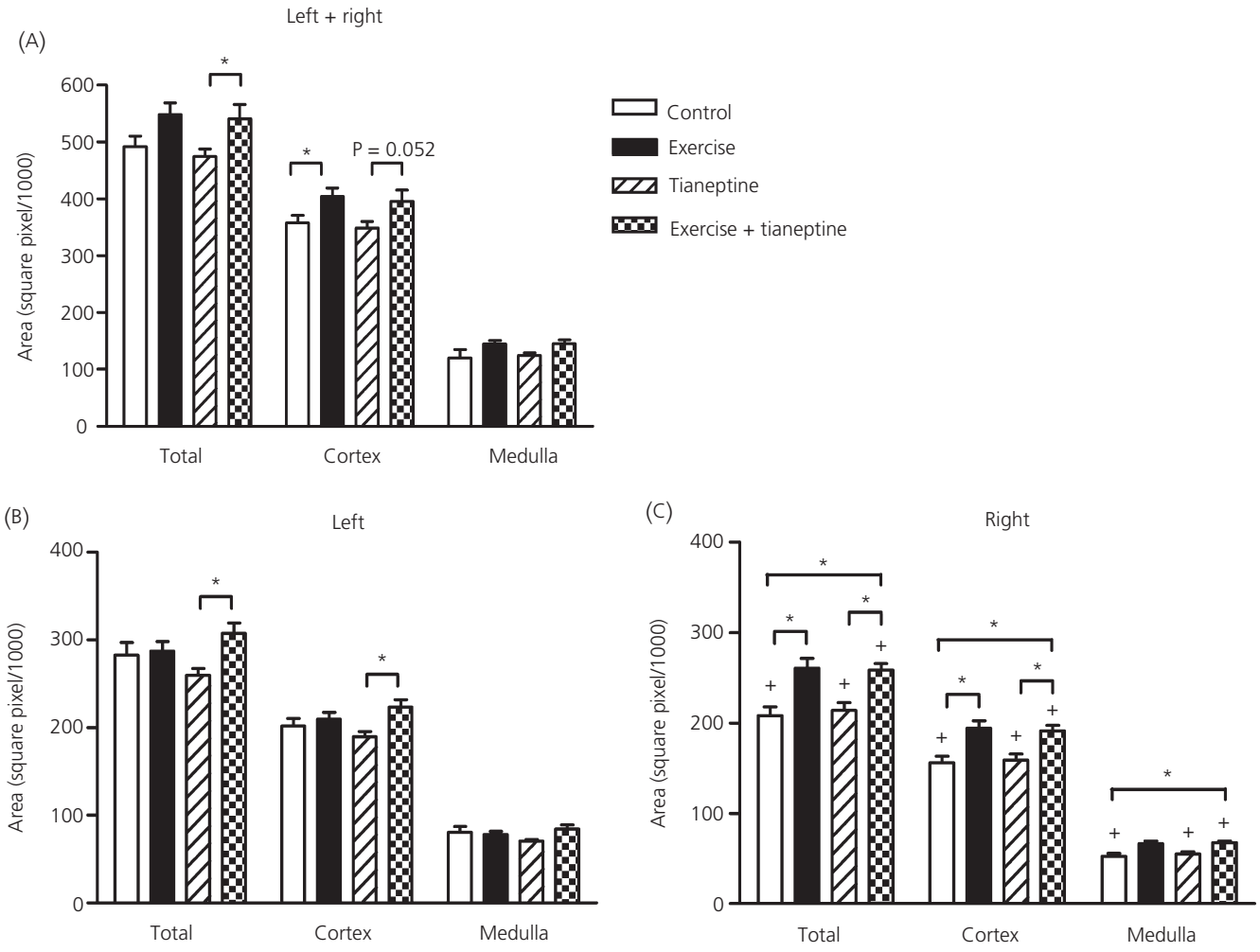

Fig. 4. Changes in the size of the left and right adrenal gland in control mice, voluntarily exercising mice, tianeptine-treated mice and tianeptine-treated exercising mice (n = 9–10 per experimental group) after an experimental period of 4 weeks. The figure shows the quantitative measurements of areas by computerised image analysis of either the entire (i.e. left + right) adrenal surface (A) or the left (B) and the right (C) adrenal gland separately. The total surface and the medullar surface were measured and, by subtraction, the cortical surface was determined. Data are presented as means ± SEM (n = 8–10). For more details, see text of Materials and Methods and the Results section. For ANOVA data, see text of the Results section; *P < 0.05, significant difference as indicated, post-hoc tests with contrasts; $^{+}$P < 0.05, significant difference between left versus right within 'total', 'cortex' or 'medulla' and within the same treatment group, post-hoc tests with contrasts.

Clearly, treatment of exercising mice with tianeptine resulted in an increased running performance of approximately 50%. Thus, in contrast to some antidepressants (31, 32), tianeptine does not appear to produce sedative effects (33, 34). Our observations also correspond with reports that tianeptine increases locomotor activity (34). Tianeptine affects serotonin levels without blocking the presynaptic uptake of serotonin (34, 35) or affecting serotonergic and α-adrenergic receptors or catecholamine uptake (34, 35). In view of the distinct effects of tianeptine on serotonin and locomotion, it is of interest to note that extracellular levels of serotonin in the brain have been found to vary in parallel with the animal's motor behaviour (36, 37). Furthermore, electrophysiological studies suggest that tianeptine inhibits currents induced by inhibitory neurotransmitters resulting in an increased excitability of serotonergic neurones in the dorsal raphe (38). Fattaccini *et al.* (23) found that acute administration of tianeptine increases 5-hydroxyindoleacetic acid, the main metabolite of serotonin, in the brainstem, cerebral cortex and striatum, which are projection areas of the dorsal raphe (39). Thus, taken together, in the present study, tianeptine may enhance running performance by affecting serotonergic mechanisms in the brain although the exact underlying mechanisms still need to be clarified.

The present study showed that the exercising mice drank more than the control animals. This may be the result of an enhanced evaporation due to physical activity induced increases in body temperature and breathing frequency. Addition of tianeptine to the drinking water also led to an increase in liquid intake in the control mice, which may be a consequence of an enhanced tianeptine-induced locomotor activity (34). However, because exercise and tianeptine acted additively on liquid intake, there appears to be no

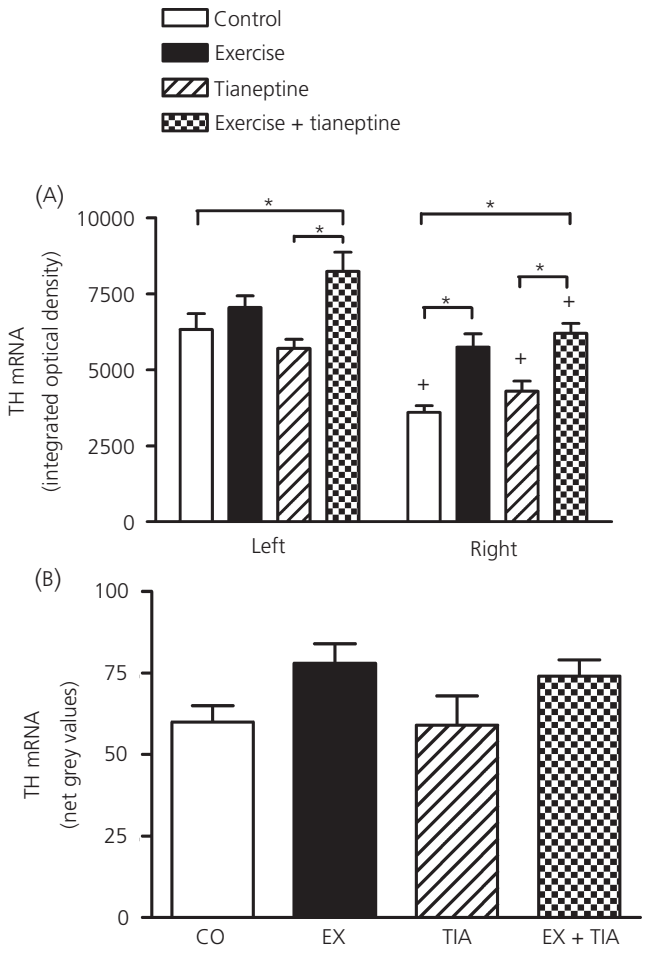

Fig. 5. Changes in tyrosine hydroxylase (TH) mRNA levels in the left and right adrenal medulla (A, n = 8–10 per experimental group) and locus coeruleus (LC) (B, n = 5–8 per experimental group) of control mice, voluntarily exercising mice, tianeptine-treated mice and tianeptine-treated exercising mice after an experimental period of 4 weeks. TH mRNA was detected by *in situ* hybridisation histochemistry and autoradiograms were analysed by computerised densitometric image analysis. In case of the adrenal medulla, TH mRNA levels are expressed as integrated optical density (i.e. net grey values × square pixels/1000). In case of the LC, TH mRNA levels are expressed as optical density (i.e. net grey values). For more details, see Materials and methods and the Results section. Data are presented as means ± SEM. For ANOVA data, see text of the Results section. (A) *$P < 0.05$, significant difference between the groups within the left or right adrenal medulla, post-hoc tests with contrasts; $^{+}P < 0.05$, significant difference between left and right adrenal medulla within the same treatment group, post-hoc tests with contrasts. (b) No significant post-hoc test results.

interaction between the performance of exercise and drug consumption on liquid intake.

An overall increase in adrenal weight is a well-known observation after exercise (40, 41). Here, we report that exercising mice show a selective increase in the size of the right adrenal cortex. Although tianeptine did not affect adrenal sizes in sedentary mice, profound changes were observed in both the left and the right adrenal cortices of antidepressant-treated exercising animals. Interestingly, although exercising mice showed only an increase in size of the right adrenal cortex, the antidepressant-treated exercising animals showed increases in both left and right adrenal cortices. The mechanisms underlying this synergism between exercise and tianeptine selectively in the left adrenal gland are presently unclear but these mechanisms, as well as those directing the growth of the right adrenal gland after exercise *per se*, may relate to changes in the neural input of these glands.

The pioneering work of M. F. Dallman and W. C. Engeland almost three decades ago has shown that neural inputs to the adrenal glands are critical growth determining factors of these glands (42, 43). The present study provides evidence that at least one of these neural inputs has increased in strength (i.e. the sympathoadrenomedullary input), as indicated by the increased expression of TH mRNA in the adrenal medulla of exercising mice. Before going into any exercise-evoked effects, it should be noted that, similar to the asymmetry in adrenal size, TH mRNA expression is also asymmetric: the left medulla expresses higher levels than the right one, suggesting that, in control mice, sympathoadrenomedullary activity is asymmetric. We found that exercising mice show increases in TH mRNA levels only in the right adrenal medulla. These observations suggest that the enlargement of the right adrenal gland after voluntary exercise may be the result of an increased sympathetic nervous input into this gland. Furthermore, the absence of size changes in the left adrenal gland of exercising animals dovetails with the unchanged sympathoadrenomedullary activity in this gland. Interestingly, the tianeptine-treated exercising mice showed large increases in TH mRNA levels not only in the right adrenal medulla, but also in the left one, strongly indicating that under these conditions sympathoadrenomedullary activity is enhanced in both adrenal glands. The reason for this bilateral increase in sympathoadrenomedullary activity may relate to the markedly enhanced running performance in the antidepressant-treated exercising animals compared to the no-drug exercising mice. Possibly, this vastly enhanced running performance requires an enhanced sympathoadrenomedullary activity to an extent where, in addition to rises in TH expression in the right adrenal medulla, rises in expression levels of this enzyme in the left adrenal medulla are also required. However, it cannot be excluded at the present time that tianeptine may directly enhance sympathoadrenomedullary capacity selectively in exercising mice thereby allowing the animals to boost their running performance. Regardless of this chicken-and-egg situation, our observation of an enhanced sympathoadrenomedullary activity in the left adrenal medulla associated with an enlarged left adrenal cortex uniquely in the tianeptine-treated exercising mice strengthens our notion that the sympathoadrenomedullary innervation is not only an important modulator of adrenocortical glucocorticoid secretion, but also a significant determinant of adrenocortical size. Furthermore, peptides such as interleukin-6 originating from the adrenal medulla may play a role in adrenocortical growth as well (44) but, presently, it is unknown whether adrenomedullar expression of interleukin-6 changes during the treatment conditions of the present study.

In addition to elevations in TH mRNA levels in the adrenal medulla after exercise, we also observed an increased expression

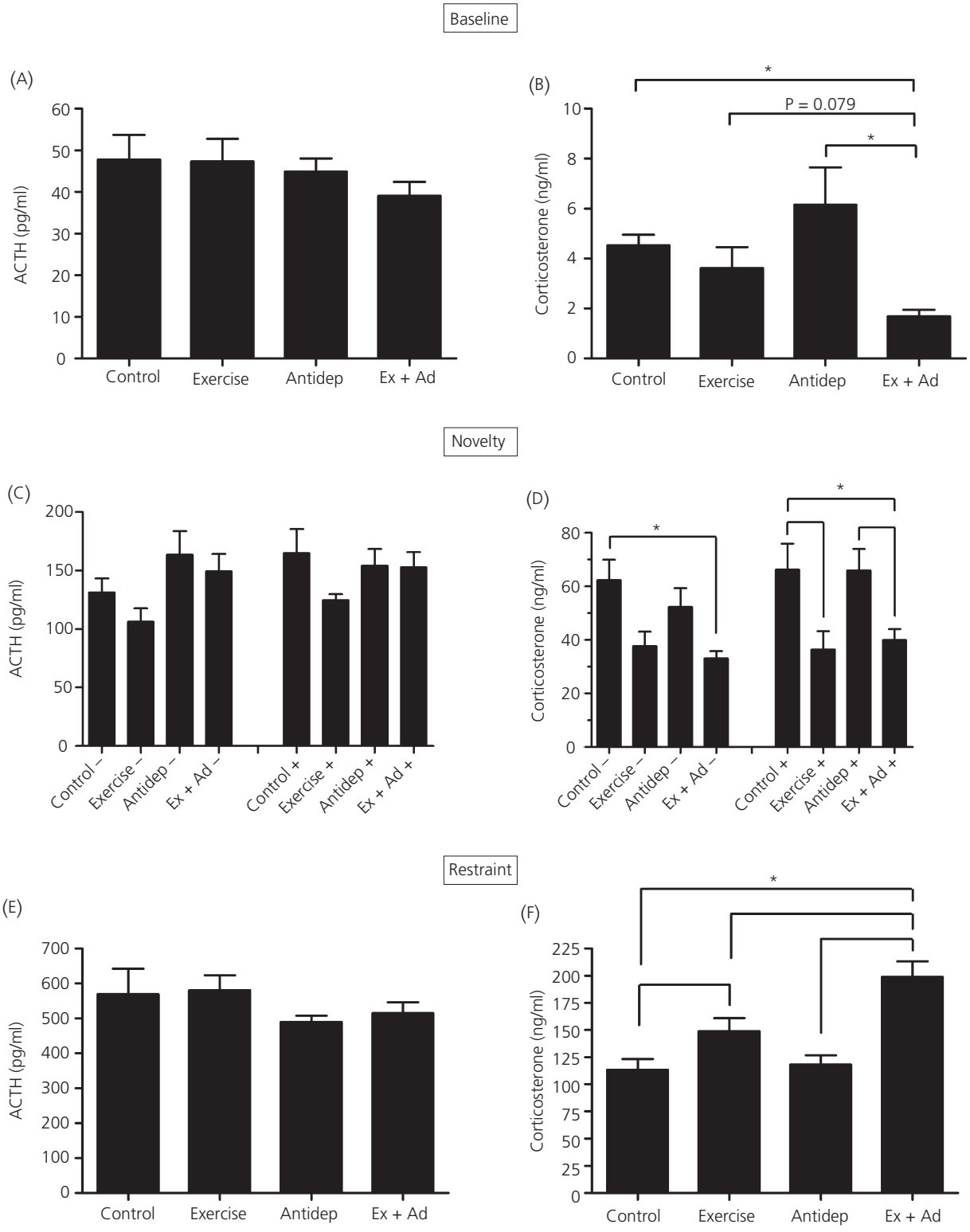

Fig. 6. Control mice, voluntarily exercising mice (Exercise), tianeptine-treated mice (Antidep) and tianeptine-treated exercising mice (Ex + Ad) were killed after an experimental period of 4 weeks either under early morning baseline conditions (A,B; n = 9–10), after 30-min exposure to a novel environment [containing a clean running wheel (+) or not (–); (C,D); n = 7–8], or after a 30-min restraint stress procedure (E,F) (n = 8–9). Plasma hormone levels [adrenocorticotrophic hormone (ACTH): A,C,E; corticosterone: B,D,F] were determined by radioimmunoassay and presented as means ± SEM. For ANOVA analysis, see text of the Results section; *P < 0.05, significantly different as indicated, post-hoc tests with contrasts.

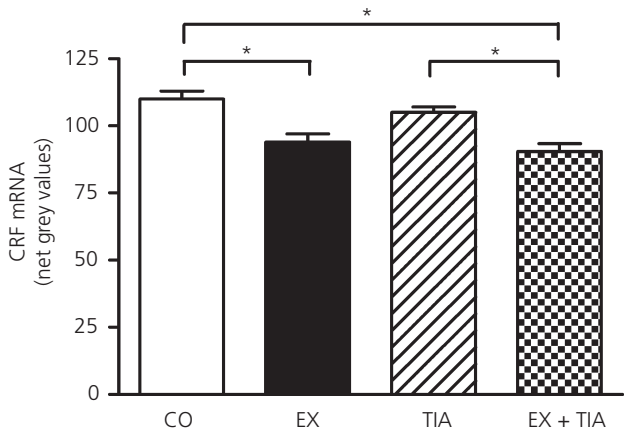

Fig. 7. Changes in corticotrophin-releasing factor (CRF) mRNA expression in the hypothalamic paraventricular nucleus of control mice (CO), voluntarily exercising mice (EX), tianeptine-treated mice (TIA) and tianeptine-treated exercising mice (EX + TIA) after an experimental period of 4 weeks. The CRF mRNA levels were determined by *in situ* hybridisation histochemistry and autoradiograms were analysed by computerised densitometric image analysis. CRF mRNA levels are expressed as optical density (i.e. net grey values; mean ± SEM, n = 5–8). For ANOVA analysis, see text of the Results section; *$P < 0.05$, significantly different as indicated, post-hoc tests with contrasts.

of this transcript in the LC. The LC plays a critical role in the autonomic, neuroendocrine and behavioural responses to stressful events. Evidence has been accumulating to suggest that changes in catecholaminergic secretory activity (mainly adrenaline) of the adrenal medulla and that of LC (noradrenergic) neurones are occurring in parallel (45, 46). The activation of the LC parallel to sympathoadrenomedullary activation appears to be brought about by circulating adrenaline (and noradrenaline) stimulating adrenergic receptors on vagal afferents, transsynaptically leading to activation of the LC (47, 48). This mechanism may explain the enhanced LC TH mRNA expression after exercise. However, the tianeptine-treated exercising mice presented a much greater overall sympathoadrenomedullar activation than the no-drug exercising animals, which was not reflected by a further enhanced TH mRNA expression in their LC. Possibly, tianeptine may have attenuated further increments in TH mRNA expression given that this antidepressant has been shown to decrease the firing rate of LC neurones in rats (49). An inhibition of LC TH mRNA expression in rats has also been shown after treatment with other antidepressants (50, 51). However, in the present study, tianeptine exerted no effects on LC TH mRNA levels in the nonexercising mice suggesting that, apparently in mice and in contrast to rats, tianeptine appears to attenuate TH mRNA expression only under stimulating conditions.

The observations of increased adrenal size of exercising mice appear to contradict the decreased baseline levels of circulating glucocorticoid levels in these animals. The contradiction was most striking in exercising animals treated with tianeptine. CRF mRNA levels in the hypothalamic PVN were also decreased in both groups of exercising mice but, in view of the unchanged plasma ACTH levels, the decreased CRF mRNA levels are unlikely to account for the diminished levels of corticosterone. With regard to the apparent paradox between the glucocorticoid levels and the size of the adrenal gland, it should be noted that the two parameters are incomparable: whereas the hormone levels are a reflection of the adrenal glands' secretory activity, the size of an endocrine gland provides only an indication of its secretory potential. Our observation that, during early morning baseline conditions, glucocorticoid levels in the tianeptine-treated exercising mice were markedly decreased, despite unchanged ACTH levels as well as enlarged right and left adrenal glands, suggests the existence of a potentiated inhibitory influence on adrenocortical glucocorticoid secretion under these conditions. Until now, the neurotransmitters tested, including adrenaline, noradrenaline, acetylcholine, vasoactive intestinal peptide and neuropeptide Y, all acted stimulatory on steroidogenesis in the inner zona fasciculata (the adrenocortical layer producing glucocorticoid hormones) (52). Thus, the identity of the inhibitory factor is still unknown.

In particular, our data on the effects of novelty and restraint on HPA hormone levels illustrate that, also with regard to stress-induced changes, the circulating corticosterone levels are shaped not only by the size of the adrenal gland and the levels of ACTH, but also by the nature of the stressor (i.e. physical versus psychological stressors), the psychological appraisal of the challenge, the activity of the sympathoadrenomedullary system, and as yet unknown factors. We observed distinct stressor-specific differences between the corticosterone responses among the different experimental groups. Restraint stress resulted in a stronger corticosterone response in both exercising groups whereas novelty exposure led to a markedly diminished response in these animals. In terms of physical and psychological nature, restraint and novelty are quite different stressors whose differential impact can be easily read from the overall higher ACTH (four- to five-fold) and corticosterone (two- to three-fold) responses in the restraint as compared to the novelty paradigm (Fig. 6C–F). Because the ACTH responses to restraint were similar between the experimental groups, it appears that the altered glucocorticoid responses were the result of changes at the adrenal level. Indeed, restraint imposes an immediate, potentially deadly threat to the animal and induces marked physical activity known to enhance sympathoadrenomedullary activity (41, 53) which, in aggregate with the elevated adrenomedullar TH mRNA expression and the enlarged (right) adrenal cortex, may explain the enhanced corticosterone responses in the exercising mice as compared to the controls. The significantly further enhanced glucocorticoid responses observed in the restrained antidepressant-treated exercising mice (as compared to the no-drug exercising animals) fit into this concept because these animals showed bilateral adrenomedullar TH mRNA elevations as well as bilateral adrenocortical enlargements. The physiological significance of these enhanced glucocorticoid responses after long-term exercise is presently unclear but high glucocorticoid levels in response to life threatening situations are thought to be beneficial with respect to the control of the host defense response and maintaining/regaining homeostasis (54–56).

The profile of corticosterone responses after a novelty challenge presented a completely different picture. Novelty is a mild psychological challenge causing anxiety because of the unfamiliarity of the new cage and the brightness of the experimental room

(500 Lux, cf. holding room: 100 Lux). It induces hardly any physical activation and therefore will barely mobilise the sympathoadrenomedullary system. A critical factor regarding the HPA axis response of the animal is its appraisal of the situation. Both drug- and no-drug-treated exercising mice showed lower glucocorticoid responses to novelty than either of the sedentary groups of animals; this is irrespective of the fact that the exercising animals had larger adrenal cortices and thus a higher glucocorticoid secretory capacity than the sedentary animals. The effect was most consistent if a running wheel was placed in the new cage which is in agreement with our previous study (12). If placed in a new cage, the exercising mice indeed ran in the wheel, thus showing displacement behaviour and thereby further reducing the emotional impact of the challenge. Recently, we have shown that long-term voluntary exercise decreases anxiety-related behaviour in mice (12, 13) which may largely explain the novelty-induced attenuated glucocorticoid responses in the exercising mice. Similar anxiety-reducing effects have been observed in humans (5) and rats [S. K. Droste, Y. Chandramohan, J. M. H. M. Reul, unpublished observations]. Thus, exercising subjects appraise a psychologically challenging event as less fear inducing than a sedentary subject, resulting in lower glucocorticoid responses. Treating exercising mice with tianeptine did not appear to change the effect of exercise on the novelty-induced corticosterone response; this is in contrast to the synergistic effects seen on restraint stress induced responses. These observations suggest that the effect of exercise on restraint and novelty induced glucocorticoid secretion involves distinct mechanisms. The mechanisms underlying the attenuating effect of exercise on the novelty-induced glucocorticoid secretion is presently unknown but may involve suppression of sympathoadrenomedullary outflow and/or the activation of an as yet unknown adrenal inhibitory factor.

In conclusion, concurrent long-term voluntary exercise and tianeptine treatment exert a potent action on the mouse HPA axis, impacting most strongly on the adrenal gland where a synergistic action was found affecting both adrenal structure and function. Whether the effects of tianeptine in conjunction with voluntary exercise were unique for this antidepressant or are universal for all antidepressants needs to be investigated in future studies. Finally, the present study shows that neural and other mechanisms at the adrenal level play a pivotal role in modulating the glucocorticoid secretory output of this endocrine organ.

Acknowledgements

We wish to dedicate this publication to Dr Mick Harbuz who died on 9 March 2006.

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