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Effects of noradrenaline and dopamine on supraspinal fatigue in well-trained men

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ABSTRACT

Purpose: Prolonged exhaustive exercise induces a failure of the nervous system to activate the involved muscles maximally (i.e. central fatigue). Part of central fatigue may reflect insufficient output from the motor cortex (i.e. supraspinal fatigue), but the cause is unresolved. To investigate the potential link between supraspinal fatigue and changes in brain concentration of dopamine and noradrenaline in temperate environment, we combined neurophysiogical methods and pharmacological manipulation of these two neurotransmitters.

Methods: Changes in performance of a cycling exercise (time trial; TT) were tested after oral administration of placebo, dopamine or noradrenaline reuptake inhibitors (methylphenidate and reboxetine, respectively) in well-trained male subjects. Changes in voluntary activation, corticospinal excitability and muscle contractile properties were tested in the knee extensors using transcranial magnetic stimulation and motor nerve electrical stimulation before and after exercise. A psychomotor vigilance task (PVT) was also performed.

Results: Compared with placebo, methylphenidate did not affect exercise performance ($P=0.19$), but more time was needed to complete the TT after administration of reboxetine (~9%; $P<0.05$). For the latter condition, the reduced performance was accompanied by a central/supraspinal fatigue (5–6%; $P<0.05$) and worsened PVT performance (7%; $P<0.05$). For the three conditions, corticospinal excitability was unchanged, and peripheral fatigue was similar. Since the ingestion of reboxetine induced a greater decrease in voluntary activation and PVT performance after the TT than placebo, with no modification in corticospinal excitability, the noradrenaline reuptake inhibitor likely affected supraspinal circuits located prior to the motor cortex.

Conclusion: These results suggest that noradrenaline, but not dopamine reuptake inhibition, contributes to the development of central/supraspinal fatigue after a prolonged cycling exercise performed in temperate conditions.

Key words : Central fatigue, EMG, transcranial magnetic stimulation, corticospinal excitability, knee extensor muscles

Introduction

Paragraph 1 Prolonged exercise or sustained contraction of a muscle group at low intensity induces a failure of the nervous system to activate the muscle at its maximal capacity (18, 36, 37). The magnitude of voluntary activation is commonly estimated by inducing a supramaximal electrical stimulus to the motor nerve during an isometric maximal voluntary contraction (MVC; 40). When extra force is evoked by the 'superimposed' stimulus, voluntary activation is considered as submaximal. A progressive deficit in voluntary activation usually develops during a prolonged exercise or contraction and is classically called central fatigue (9, 40). Furthermore, studies applying transcranial magnetic stimulation (TMS) to motor cortical areas during an MVC suggested that, when the stimulation evokes an additional torque increment, part of the central fatigue can be due to an insufficient output from the motor cortex (9, 17, 36, 37, 38, 40). This deficit is usually called supraspinal or cortical fatigue.

Paragraph 2 Although the underlying mechanisms are not yet understood, it has been suggested that central fatigue might be related to a change in the synthesis and metabolism of brain monoamines, such as serotonin, dopamine and noradrenaline (for review; see 35). These neurotransmitters play a key role in the modulation of various brain functions such as motivation, arousal, attention, stress responses, and motor control (2). For example, it has been suggested from animal studies that an increased serotonergic activity (29) and the ratio of serotonin to dopamine brain content during exercise (6) may be important for the development of central fatigue. However, several pharmacological studies performed in humans failed to alter the exercise capacity through changes in serotonergic neurotransmission (32), suggesting that the role of serotonin is often overestimated and dependent on drug dosages and receptor that is targeted (25). Other investigations reported that pharmacologically induced inhibition of the reuptake of dopamine and noradrenaline led

to changes in performance in both normal (12, 33) and high ambient temperatures (30, 33, 34). However, to our knowledge, no studies have investigated whether the effects of these drugs on the performance of a prolonged exercise are related to changes occurring at central/supraspinal level.

Paragraph 3 In humans, it is impossible to directly measure the effects of specific brain neurotransmitters on motor cortical excitability and output. Therefore, we pharmacologically manipulated the concentration of dopamine and noradrenaline to investigate the potential effects of these two brain neurotransmitters on central/supraspinal fatigue. Depending on the reseach field, supraspinal fatigue (38, 40), sometimes called brain or cortical fatigue (22), has been reported as an alteration affecting not only the motor cortex (38, 40) but also a fronto-parietal network involved in cognitive functions (22). These two aspects of fatigue can be investigated by measuring voluntary activation (9, 17, 38, 40) and assessing cognitive performance (22, 24) in the same experiment. Accordingly, electrophysiogical methods (voluntary activation tested by TMS) and a vigilance task (Psychomotor Vigilance Task or PVT; 22) were used to explore further the supraspinal component of central fatigue shortly after a prolonged cycling exercise performed in temperate environment. We hypothesized that if there is a supraspinal component in central fatigue, the effects would affect not only the motor output, but also the cognitive function.

Material and methods

Subjects

Paragraph 4 Ten well trained cyclists or triathletes (age 27 ± 6 yr; height 1.81 ± 0.05 m; mass 75.7 ± 8.0 kg; maximal power output (Wmax) 367 ± 23W; VO_2max 67 ± 7 ml/min/kg) participated in this investigation. In the present study, a fatiguing exercise involving most of the lower limb muscles was preferred to an isometric contraction of an isolated muscle group

as it induces a much longer depression in voluntary activation (27, 36). The time trial protocol applied (see description below) has been shown to be much more reliable and reproducible than a constant load exercise sustained to exhaustion, especially in well-trained subjects (14). Therefore, the subjects selected for this experiment had an experience of at least five years of regular cycling training and competition that ensured a high degree of reproducibility of performance across the different trials (14, 32, 33, 34). Because of potential issues with the menstrual cycle and hormonal variations in female subjects, experiments were only performed on a male population.

Paragraph 5 Before the beginning of the study, all volunteers received written information regarding the nature and purpose of the experimental protocol. Following an opportunity to ask questions, a written statement of consent was signed. The experimental protocol was approved by the Ethics Committee of the Vrije Universiteit Brussel (Brussels, Belgium) and experiments were performed in accordance with the Declaration of Helsinki.

Cycling exercise

Paragraph 6 Prior to the experimental sessions, all subjects completed a maximal exercise test which consisted of a continuous incremental cycling exercise. Subjects started the maximal exercise test at a resistance of 80W, every three minutes 40W was added until volitional exhaustion, i.e. the moment that subjects could not maintain 80 RPM. This test was used to determine the power output required to elicit 55% and 75% Wmax and VO_2max. The experimental cycling protocol was identical to the one used previously (33, 34). Subjects completed a familiarization trial, followed by three experimental trials, all separated by at least 5 days to ensure full recovery. The first part of each trial consisted of 60 min fixed intensity exercise at a workload corresponding to 55% Wmax, and was followed by a time trial (TT) to measure performance. There was a 1–2 min delay between the end of the

constant load exercise and the beginning of the TT, to program the ergometer (Lode Excalibur Sport, Groningen, the Netherlands). The TT required the subjects to complete a predetermined amount of work equal to 30 min at 75% Wmax as quickly as possible (14). Subjects began the TT at a workload corresponding to 75% Wmax, but were free to increase or decrease their power output as desired from the outset. During the TT, a computer program displayed a bar indicating the percentage of total work completed to give the subject an indication of his progress. Throughout the protocol, no feedback was provided regarding time lapsed, power output, pedal cadence or heart rate. During exercise subjects had *ad libitum* access to plain water. Ratings of perceived exertion (RPE; scale from 6 to 20; 4), and heart rate were assessed during the TT. Core temperature was measured before and during the cycling exercise.

Drugs

Paragraph 7 Subjects were randomly assigned to one of three drug treatments. The first treatment consisted of 8 mg reboxetine (Rebox; noradrenaline reuptake inhibitor) on the night before the experiment. On arrival in the lab, subjects ingested another 8 mg Rebox. In the second condition, subjects ingested a placebo (lactose; 20 mg) on the night before the experiment and 40 mg of methylphenidate (Mph; dopamine reuptake inhibitor) on arrival in the lab. Mph is an amphetamine-like stimulant that is widely administered to treat children with attention deficit hyperactivity disorder (31). It is currently on the doping list of the World Anti-Doping Agency (WADA), indicating athletes can only use it under the condition of therapeutic use exemption. The third treatment was the placebo (Pla), both the night before and the day of the experimental trial subjects ingested 20 mg lactose. The treatment was randomized and administered in a double-blind cross-over manner. Rebox, Mph and Pla

capsules were prepared by an independent pharmacy to appear indistinguishable with regard to dimensions and color.

Ergometric device

Paragraph 8 The neuromuscular parameters were recorded while the subjects sat on an adjustable chair such that the hip and knee joints were at 100° (angle between trunk and thigh) and 80° (full extension = 0°), respectively. The chair had a long back-rest to provide full back support. The head of the subjects was secured in a custom-made headrest to ensure a stable position during the experiment. The right leg of the subjects was connected to a force transducer placed ~2 cm above the lateral malleolus using velcro straps. To minimize the movement of the trunk during contractions, the subject was attached to the chair by a racing harness. The position of the headrest and force transducer as well as the harness adjustments were recorded for each subject to ensure identical position in each experimental session.

Torque and electromyographic recordings

Paragraph 9 The isometric torque developed by the knee extensor muscles was measured by a force transducer (linear range, 0–2,500 N; U2000 load cell, Maywood Instruments Ltd, Basingstoke, UK) connected to the leg and the chair by a rigid system. The signal from the force transducer was amplified by a custom-made amplifier. Voluntary and electrically evoked electromyographic (EMG) activities were recorded from the rectus femoris, the vastus medialis and the long head of the biceps femoris by means of self-adhesive bipolar electrodes (Ag-AgCl, 10-mm diameter, interelectrode distance ~5 cm). The EMG electrodes were placed over the muscle belly of each muscle; ~10 cm proximal to the superior border of the patella for the rectus femoris and on the mid-part of the muscle belly, determined by manual testing, for the vastus medialis and the long head of the biceps femoris.

The ground electrodes were located over the lateral condyle of the tibia. The position of the electrodes was marked with indelible ink to ensure a similar location on the different visits. All EMG signals were amplified (× 1,000) and filtered (10 Hz to 1 kHz) by a custom-made differential amplifier. The EMG and torque signals were acquired on a personal computer at a sampling rate of 2 kHz with a data-acquisition system and analyzed off-line with AcqKnowledge analysis software (Model MP 100, Biopac Systems, Santa Barbara, CA, USA).

Paragraph 10 The potential effect of EMG crosstalk between knee extensor muscles and biceps femoris was evaluated in four subjects by recording the EMG activity of biceps femoris, vastus medialis and rectus femoris muscles during a maximal electrical stimulation of the latter one at its motor point. To quantify crosstalk, the ratio between the amplitude of the signal recorded over biceps femoris or vastus medialis and the stimulated muscle was then computed. The EMG amplitude of the biceps femoris and vastus medialis were respectively ≤ 4 and 6% of the rectus femoris amplitude indicating that cross-talk between synergist and antagonist muscles was negligible in our experimental conditions.

Electrical stimulation

Paragraph 11 The knee extensors were stimulated by single supramaximal electrical pulses (0.2-ms duration) delivered by a constant current stimulator (DS7A Digitimer, Welwyn Garden City, UK) that was triggered by a digital timer (Master-8, AMPI, Jerusalem, Israel). The stimuli were delivered to the femoral nerve through two self-adhesive electrodes (Ag-AgCl, 10-mm diameter). The cathode was positioned over the nerve in the femoral triangle, and the anode was placed midway between the greater trochanter and the iliac crest (36). The precise location of the cathode was determined at rest by weak electrical stimulation whereas the maximal stimulus intensity was adjusted during submaximal contractions (~50% MVC).

The level of stimulation was then set 30% above that required to induce a Mmax during the submaximal contractions.

Transcranial magnetic stimulation

Paragraph 12 A double-cone coil (130-mm outer diameter) was positioned over the cortex to elicit motor evoked potentials (MEPs) in the right knee extensors with a Magstim 200 stimulator (Magstim, Dyfed, UK). The junction of the double cone coil was positioned 1–2 cm to the left of the vertex. The direction of current flow in the coil preferentially activated the left motor cortex. At the beginning of each experiment, the coil position was determined to stimulate optimally the rectus femoris and vastus medialis muscles and this position was marked on the scalp. The stimulator output (30–60% of maximum) was set to evoke: large MEP in both rectus femoris and vastus medialis (>80% of Mmax area at 50% MVC), minimal MEP in the biceps femoris, and the greatest superimposed twitch during the different target torques used in the protocol (see below). The custom-made headrest ensured a stable location of the coil on the scalp during the experiment.

Psychomotor Vigilance Task

Paragraph 13 The psychomotor vigilance test was used as a sustained attention task in present study (22). For a detailed description of the task, readers are referred to the original study by Dinges and Powell (8). Briefly, a laptop computer displayed a visual stimulus (fireball, always in the middle of the screen) at a variable interval (between 2-10 s). Subjects were required to respond by pressing the space-bar with the index of the dominant hand. The distance between the subject's eyes and the screen was ~40 cm. Subject's reaction time was displayed on a computer screen for 1s after space-bar pressing. If the subject did not respond

within 500 ms, the trial was stored as a lapse, and a warning message appeared to the participant.

Paragraph 14 The PVT was performed once before and once 15 min after the end of the cycling exercise when the subject was sitting on the chair used for the recording of the neuromuscular parameters (see description above). The PVT ran for a total period of 10 min (100 stimuli). The reaction time of correct responses (<500 ms) and number of lapses were stored.

Experimental protocol

Paragraph 15 As already mentioned, all subjects participated in a familiarization session and three experimental sessions (Pla, Rebox and Mph). The familiarization trial was undertaken to ensure that subjects were accustomed to the procedures employed during the investigation and to minimize any potential learning or anxiety effects. During this session, the subjects practiced the isometric contractions and experienced the electrical motor nerve stimulation and TMS. The familiarization trial was identical to the experimental trials in all respects.

Paragraph 16 Experimental trials were undertaken in temperate (18^{o}C) conditions with relative humidity maintained between 50–60%. Subjects were instructed to record dietary intake and physical activity during the two days before the first trial, and to replicate this in the two days prior to the subsequent experimental trials. No exercise, caffeine or alcohol consumption was permitted in the 24 hours before each trial. For each subject the four sessions were performed at the same time of the day, either at the end of the morning or in the afternoon. For all trials, subjects were dressed in cycling shorts, socks and shoes.

Paragraph 17 On arrival in the lab, subjects inserted a rectal thermister (Gram Corporation LT-8A, Saitama, Japan) 10 cm beyond their anal sphincter for the measurement

of core temperature and heart rate was recorded telemetrically by a cardio frequency meter (Polar Accurex plus, Kempele, Finland). They were then placed in the experimental chair to record the neuromuscular parameters. After a warm up period consisting in a few submaximal contractions, subjects performed 2-3 brief MVCs (~2–3 s) to determine their baseline maximal torque. The torque of the greatest MVC was then measured, and two submaximal target torques (50 and 75% MVC) were determined and displayed on a computer screen used as visual feedback. Thereafter, pre-exercise recordings were started. Voluntary activation was tested by TMS and motor nerve stimulation. To that end, subjects performed sets of brief contractions. Each set was composed of a brief MVC followed by contractions at 50% and 75% MVC, and ended by an MVC. TMS was delivered during the first three contractions whereas motor nerve stimulation was delivered during the last MVC. Subjects were asked to maintain the required contraction level during and immediately after TMS or motor nerve stimulation. The resting potentiated twitch was recorded in response to motor nerve stimuli delivered 3–5 s after the last MVC (Figure 1). The order of the contractions at the different intensities (50, 75% and 100% MVC) and of the two stimulation types (TMS and electrical stimulation) was randomized across subjects but was kept constant for each subject across experimental sessions. For the pre-exercise recordings, three sets of contractions were at least performed (Figure 1). In each set, the contractions were separated by 10 s rest, and sets were separated from each other by 4 min rest. The TMS protocol, originally introduced by Todd and colleagues (40), has been shown to produce reliable estimations of resting twitch and voluntary activation in the knee extensor muscles (11, 36).

[FIGURE 1]

Paragraph 18 After the cycling effort, two sets of contractions and stimulations similar to the pre-exercise condition were performed at 10, 30 and 45 min of recovery (R10,

R30 and R45, respectively; Figure 1). For all contraction levels and stimulation types, the recording was repeated when the torque level did not remain constant during or just after the time at which the stimulation was delivered. The PVT was performed 15 min after the end of the TT.

Data analysis

Paragraph 19 The duration of the TT and the time course of the power output during the TT were measured. For the trial that produced the greatest MVC torque, the maximum torque and associated average EMG activity (aEMG) of rectus femoris, vastus medialis and biceps femoris, recorded before and after the cycling exercise, were determined for a 500-ms period during the MVC torque plateau. The peak-to-peak amplitude and area of the MEP, and the Mmax recorded during the contractions at different intensities were also measured. As neither MEP amplitude, nor MEP area changed after the cycling exercise, only areas are reported. To avoid the influence of activity-dependent changes in the muscle fiber membrane during the fatiguing contraction and to assess the corticospinal adjustments, MEP area was normalized, at each time point, to the corresponding Mmax area recorded during the MVC. The MEP latency and duration of the silent period (SP) following TMS were measured in both the rectus femoris and vastus medialis. Latency was defined as the time between the stimulus artifact and the first major deflection in the EMG signal. The SP duration was determined in the contracting muscle as the interval from the stimulus artifact to the return of continuous EMG activity. The superimposed twitch amplitude, recorded in response to TMS or motor nerve stimulation, was measured as the difference between the superimposed peak torque minus the MVC torque. Peak torque, time-to-peak torque and half-relaxation time of the resting twitch, in response to motor nerve stimulation, were also measured. For each subject and time point, parameters recorded from multiple sets were averaged. The amplitude

of the resting twitch evoked by TMS was estimated rather than measured directly (see 40). For each subject, a linear regression of the amplitude of the superimposed twitch evoked by TMS against voluntary torque was performed for the 50, 75 and 100% MVC contractions. The *y*-intercept was taken as the amplitude of the estimated resting twitch (ERT; 40). For the two techniques, voluntary activation level (% of maximum) was calculated according to the following equation: [1 – superimposed twitch/resting twitch (or ERT)] × 100 (36, 40). For the PVT, reaction time and lapses averages before and 15 min after the cycling exercise were compared for each subject.

Statistical Analysis

Paragraph 20 Prior to comparing each dependent variable, the normality of the data was confirmed with the Kolmogorov-Smirnov test. To evaluate differences in time necessary to complete the TT and in mean power output during the TT in the three conditions (Rebox, Mph and Pla), a one-way ANOVA with repeated measures was used. Data collected over time before, during and after the cycling exercise were analyzed using a two-factor (drug x time) ANOVA with repeated measures. When a significant main effect was found, a Tukey post hoc test was used to identify the significant differences among the selected means. The relation between subjects' change in MVC torque and voluntary activation tested by TMS 10 min after the TT was fitted by a linear regression using the least-squares method. For all comparisons, statistical significance was accepted at $P<0.05$. Data are reported as means ± SD within the text, and means ± SEM in the figures.

Results

TT performance

Paragraph 21 All subjects completed the three experimental trials. They took 9.4% more time to complete the TT in the Rebox condition (33.66 ± 3.61 min) compared with the

Pla condition (30.78 ± 2.08 min; P<0.05). Despite a one minute or 3.4% faster TT performance, the time needed to complete the TT was not significantly different between Mph (29.73 ± 1.40 min) and Pla trials (P=0.19). The mean power output generated during the TT was significantly (P<0.05) lower in the Rebox trial compared with the Pla trial (252 ± 31 *vs.* 271 ± 26 W) while no difference (P=0.18) was observed between Mph (279 ± 28 W) and Pla trials. In Pla and Mph conditions, the power output remained constant throughout the TT while it decreased progressively for the Rebox trial (ANOVA drug x time interaction: P<0.01; Figure 2A).

Paragraph 22 Subjects' RPE at the end of the TT was similar between Pla (18.0 ± 1.4), Rebox (18.1 ± 1.1) and Mph (18.7 ± 1.1) conditions (P=0.18). Heart rate was higher for Mph (185.4 ± 7.3 bpm) than for Pla (174.4 ± 9.2 bpm; P=0.05) at the end of the TT, but there was no significant effect for Rebox (168.8 ± 16.6 bpm; P=0.40). At the completion of the TT, core temperature was significantly greater (P<0.001) compared with pre-exercise values for all conditions (37.1 ± 0.2, 37.1 ± 0.3, and 37.3 ± 0.2°C for Pla, Rebox and Mph, respectively). It was slightly greater after Mph administration (39.4 ± 0.5°C; P<0.05) but was not significantly influenced by Rebox (38.8 ± 0.3°C; P=0.64) when compared with Pla (38.9 ± 0.2°C). Core temperature remained significantly elevated 10 min after the end of the TT for Pla (38.5 ± 0.5°C), Rebox (38.4 ± 0.4°C) and Mph (38.9 ± 0.5°C; post hoc tests: P<0.001) and did not return to control values till the end of the recovery period (post hoc tests: P<0.01).

MVC torque and aEMG

Paragraph 23 Before the cycling exercise, average MVC torque was similar (P=0.87) for Pla, Rebox and Mph trials and corresponded to 233.3 ± 65.5, 236.0 ± 73.3 and 232.8 ± 67.4 Nm, respectively. When recorded 10 min after the end of the TT, the MVC torque was significantly reduced for the three conditions (post hoc tests: P<0.05; Figure 2B). This

reduction tended to be slightly greater for Rebox as compared to Pla and Mph trials (P=0.15; Figure 2B). For the three conditions, the deficit in MVC torque was still present after 45 min of recovery (post hoc tests: P<0.05).

Paragraph 24 As for the MVC torque, the corresponding aEMG for the agonist and antagonist muscles were also similar in pre-exercise conditions regardless of the drug administrated (P>0.37). The aEMG values in Pla, Rebox and Mph conditions were, respectively, 709.6 ± 234.1, 716.1 ± 245.4 and 693.2 ± 299.0 μV for rectus femoris, 778.6 ± 448.6, 771.9 ± 369.1 and 753.2 ± 366.1 μV for vastus medialis and 149.2 ± 60.0, 126.0 ± 49.5 and 159.2 ± 64.9 μV for biceps femoris. There was no statistically significant interaction between drug and time for the aEMG change following the cycling exercise (P=0.19). The aEMG of the agonist muscles averaged across the recovery period decreased slightly for the three conditions (91.7 ± 8.2, 88.8 ± 9.1, 95.9 ± 9.2% of control values for Pla, Rebox and Mph, respectively). However this decrease was significant for Pla and Rebox conditions (P<0.05) but not for the Mph condition (P=0.28). A similar tendency was observed for the aEMG of biceps femoris (93.8 ± 18.1, 89.2 ± 24.6, 94.1 ± 25.5% of control values for Pla, Rebox and Mph, respectively) but the reduction did not reach statistical significance (post hoc tests; P>0.18).

[FIGURE 2]

MEP parameters

Paragraph 25 The MEP area (% Mmax) recorded in rectus femoris and vastus medialis during the MVC before the cycling exercise was similar in the three conditions (P=0.25; Table 1). Regardless the drug administrated, it was unchanged in both rectus femoris (ANOVA time effect: P=0.78) and vastus medialis (ANOVA time effect: P=0.81) when measured 10 min after the end of the TT. The MEP recorded from the biceps femoris was

very small compared with that from the knee extensors and similar for the three conditions (9.8 ± 4.2, 9.6 ± 5.5, 9.3 ± 3.3% of knee extensors MEP values for Pla, Rebox and Mph, respectively; P=0.95). It did not change after the cycling exercise (ANOVA time effect: P=0.56).

Paragraph 26 The MEP latency and SP duration, measured in rectus femoris and vastus medialis, were similar during pre-exercise recordings for the three conditions (P>0.8) and did not change after the cycling exercise (ANOVA time effect: P>0.25; Table 1). Regardless the drug administrated, the Mmax area recorded during the MVC was not statistically different before exercise for rectus femoris (P=0.32) and vastus medialis (P=0.11), and unchanged when measured 10 min after the cycling exercise compared with control values (ANOVA time effect, P=0.53 and P=0.49, respectively; Table 1).

[TABLE 1]

Voluntary activation

Paragraph 27 During pre-exercise recordings, voluntary activation measured with TMS was not statistically different for the three conditions and represented 96.9 ± 3.0, 94.3 ± 8.2 and 96.6 ± 2.8% of maximal activation for Pla, Rebox and Mph, respectively (P=0.34; Figure 3C). There was a significant ANOVA interaction between drug and time (P<0.05) and post-hoc tests indicated that voluntary activation was significantly reduced in the Rebox condition (5.4%; P<0.001) but unchanged for Pla and Mph conditions (P=0.7 and P=0.9, respectively) when measured 10 min after the end of the cycling exercise (Figure 3C). For the Rebox trial, this reduction persisted till 45 min of recovery (P=0.2).

Paragraph 28 A significant linear association ($y = 0.83x + 16.56$; $r^2 = 0.29$) between subjects' voluntary activation tested by TMS and change in MVC torque 10 min after the end of the TT was found when data from the three conditions were plotted together (P<0.01).

[FIGURE 3]

Paragraph 29 During pre-exercise recordings, voluntary activation tested by motor nerve stimulation was similar (P=0.96) for Pla, Rebox and Mph trials (94.2 ± 2.6, 94.4 ± 3.9 and 94.6 ± 5.0%, respectively; Figure 3D). For the three conditions, it was slightly reduced 10 min after the end of the cycling exercise but again, the difference was only statistically significant for the Rebox condition (4.7%, P<0.05; Figure 3D). Although still present after 30 min of recovery, the deficit in voluntary activation was no more statistically different (P=0.07) from control value.

Twitch contractile properties

Paragraph 30 The peak torque of the twitch induced by motor nerve stimulation at rest did not differ statistically between the three conditions during pre-exercise recordings (P=0.11; Table 2). Compared with control values, it was significantly decreased shortly after the TT in the three conditions (post hoc tests: P<0.05; Table 2). There was, however, no significant ANOVA interaction between drug and time (P=0.4). Twitch torque was still significantly lower than control values after 45 min of recovery (post hoc tests: P<0.05). As for peak torque, the twitch time-to-peak torque and half-relaxation time were similar for the three conditions during pre-exercise recordings (P=0.18 and 0.73, respectively; Table 2). Ten min after the end of the TT, all these values were reduced for Pla, Rebox and Mph (post hoc tests: P<0.05; Table 2). The twitch time-to-peak torque recovered during the following resting period and did not differ statistically from control value after 30 min of recovery for Mph (P=0.21) and after 45 min for Pla (P=0.07) and Rebox (P=0.63). Half-relaxation remained below the initial values at the end of the recovery period for Pla (P<0.01) and Mph (P<0.05) but not for Rebox (P=0.27).

[Table 2]

Paragraph 31 As for the electrically-induced twitch, the ERT was similar before exercise in the three conditions (34.5 ± 14.6, 36.9 ± 18.7 and 37.0 ± 22.4 Nm for Pla, Rebox and Mph, respectively; P=0.6) and was also similarly reduced (post hoc tests: P<0.05) at the end of the TT in the three conditions (70.5 ± 19.9, 65.5 ± 23.0 and 69.9 ± 21.7 % of control values for Pla, Rebox and Mph, respectively). There was no ANOVA interaction between drug and time (P=0.78). ERT was still significantly lower than control values after 45 min of recovery (post hoc tests: P<0.05).

PVT

Paragraph 32 The control values for the reaction time and number of lapses were similar regardless of the drug administrated (P=0.73 and 0.40 for the reaction time and number of lapses, respectively; Table 3). For the reaction time, the ANOVA showed a significant interaction between drug and time (P<0.05). It increased significantly for the Rebox condition (P<0.01), did not change for the Pla trial (P=0.97) and decreased for the Mph trial (P<0.01; Table 3). In contrast, the number of lapses did not change significantly after the cycling exercise for any condition (ANOVA time effect: P=0.48; Table 3).

[Table 3]

Discussion

Paragraph 33 The objective of the current study was to investigate the potential link between neural fatigue and changes in brain concentration of noradrenaline and dopamine by combining neurophysiogical methods and pharmacological manipulation of these two neurotransmitters in a temperate environment. To that purpose, we tested the effects of oral administration of noradrenaline and dopamine reuptake inhibitors on acute cycling

performance and central/supraspinal fatigue. The main novel finding is that the decrease in cycling performance induced by the noradrenaline reuptake inhibition was accompanied by a greater central/supraspinal fatigue, and a worsened PVT performance. In contrast, the dopamine reuptake inhibition did not influence the TT performance and central/supraspinal fatigue differently than the placebo in the current environmental conditions.

TT performance, MVC torque and twitch contractile properties

Paragraph 34 TT performance was decreased in Rebox condition whereas Mph had no significant influence on performance. These results are in agreement with previous studies performed in our laboratory during similar cycling exercise and environmental conditions (33, 34). Whereas the TT performance was not influenced by Mph in normal ambient temperature (18°C), it was improved by 16% in the heat (30°C; 34). The lack of effect of Mph in temperate conditions was confirmed, even though the dose of Mph has been doubled (40 vs. 20 mg) compared with our previous investigation (34).

Paragraph 35 The decline in MVC torque measured 10 min after the end of the TT was lower than what is usually reported for moderately trained subjects after similar cycling efforts (18, 36) but comparable to well-trained cyclists or triathletes (20, 28). The mean decrease in MVC torque was slightly greater for Rebox than Pla trial. However, this difference did not reach statistical significance. This observation may be partly explained by the modest fatigue-related decline in MVC torque in the three conditions and the relatively high inter-subject variability. However, when individual data of the three conditions were plotted together, a significant linear association ($r^2 = 0.29$; $P<0.01$) was observed between subjects' voluntary activation tested by TMS 10 min after the TT and changes in MVC torque. This observation suggests that the level of voluntary activation measured shortly after the TT, and which is influenced by the drug administrated, contributed partly to reduce MVC torque.

The relatively greater reduction in TT performance compared with the loss of MVC torque under Rebox condition could be due to the delay required (10 min) to replace very precisely the subject on the experimental setup used to test the electrophysiological parameters after the end of the cycling exercise. During such time period some recovery may have occurred. Furthermore, the contribution of neural factors (see below) to the alteration in performance may be more pronounced for a multi-joints task such as cycling than for a single joint isometric MVC that requires less complex muscular synergy.

Paragraph 36 The decrease of the electrically-induced resting twitch and ERT observed in our study indicates that impairment of the excitation-contraction coupling contributes to the fatigue observed after prolonged cycling (18, 20, 36). The similar decrease in twitch torque and time course for the three conditions suggests that peripheral fatigue induced by the cycling exercise was not worsened by the noradrenaline reuptake inhibitor. Consequently, the negative influence of Rebox on TT performance should be mainly due to neural impairments. The shortened twitch time course (time-to-peak torque and half-relaxation time) was most probably related to the increase in core temperature (5) and the relative interaction between fatigue and postactivation potentiation (10).

Neural contribution to muscle fatigue

Paragraph 37 Pre-exercise voluntary activation values measured by supramaximal electrical stimulation of the motor nerve and TMS were similar in the three conditions. The cycling effort did not significantly reduce the voluntary activation level tested by electrical stimulation for Pla and Mph trials. The absence of central fatigue in the Pla condition is surprising since, contrary to sustained isometric fatiguing contractions of an isolated muscle group after which voluntary activation usually recovers within the first minutes (15), a long-lasting central and supraspinal fatigue has been reported after prolonged exhaustive exercises

(27, 36). Our results may however be explained by the high training level of our subjects as it has been reported that well-trained athletes did not display significant central fatigue when tested within a 30 min period after prolonged cycling exercise (20, 28).

Paragraph 38 In contrast to the Pla trial, a significant central fatigue was observed in the Rebox condition. Indeed, we observed a similar deficit in voluntary activation when tested 10 min after the TT by TMS and motor nerve stimulation. Although both TMS and motor nerve stimulation measure the failure of the nervous system to drive the muscle maximally during fatigue, TMS probes more specifically the supraspinal component of central fatigue (36, 38, 40). A direct quantitative comparison of the two techniques is not straighforward because they can activate synergistic muscles differently and the shapes of the voluntary activation–force relations for motor cortical and motor nerve stimulation differ slightly (38, 40), however, the reduction in voluntary activation tested by TMS indicates that a long-lasting (30 min) supraspinal fatigue was present after the cycling exercise. The presence of a deficit in activation due to supraspinal impairment shortly after the TT in the Rebox condition suggests that noradrenaline is an important neurotransmitter in the modulation of central/supraspinal fatigue under the present environmental conditions. The absence of significant effect of Mph on central/supraspinal fatigue in our experimental conditions does not exclude, however, a role for dopamine in central fatigue in high ambient temperature (30, 34). Previous studies have indeed shown that mechanisms involved in central fatigue might be temperature dependent (30). Together this hypothesis and the current results indicate that future research should focus on the effects of the different neurotransmitter systems in high ambient temperature that would increase the thermal stress to a much greater extent than in the current study.

Location of neural impairments and possible mechanisms

Paragraph 39 As the normalized MEP area recorded during the MVC did not change following the cycling exercise, the responsiveness of neurons in the pathway from the motor cortex to the muscle was presumably not impaired (21, 36). Similarly, as the SP duration was not affected for the three conditions when tested shortly after the cycling exercise, a change in intracortical (13) or/and motor neuronal inhibition is unlikely (38).

Paragraph 40 The PVT is considered as a reliable method to measure "cognitive fatigue", defined as "vigilance decrement," or "time on-task effect" (24), consequent for example to sleep deprivation or during taxing cognitive tasks (22). Our observation of a greater PVT performance after exercise in the Mph condition is consistent with studies showing that this drug can improve performance in vigilance tasks in healthy subjects and patients with attention deficit disorder (31, 39). In contrast, Rebox had a negative effect on PVT performance after exercise. The changes in reaction time observed after the end of TT in Rebox and Mph conditions cannot be ascribed to a change in motor time because MEP latency and twitch time-to-peak torque displayed a similar time course in the three conditions. Since Rebox in resting conditions has no significant effect on reaction time during psychometric tests in healthy subjects (16), it is suggested that the noradrenaline reuptake inhibitor and exercise interact at the supraspinal level and affect brain centers located prior to the motor cortex.

Paragraph 41 Animal studies have shown that the locus coeruleus, which contains the neurons synthesizing noradrenaline, provides numerous projections to different parts of the central nervous system involved in behavioral, attention, arousal and vigilance regulation (for a review; see 3). Recent works indicate that noradrenaline contributes to maintain forebrain neuronal activity states appropriate for sensory information integration (e.g. waking) and modulates the collection and processing of salient sensory information (3) through its projections to cortical and subcortical sensorimotor, attention, and memory circuits (3, 19). In

addition, iontophoretically applied noradrenaline in anesthetized animals can enhance both excitatory and inhibitory responses of somatosensory cortical neurons to afferent synaptic input (42). This last effect was selective for noradrenaline since dopamine does not exhibit a similar property (41).

Paragraph 42 The noradrenaline reuptake inhibitor we administrated may thus have affected the integration of sensory information or/and motor cortical sensitivity to these sensory afferents and consequently altered the generated motor output (1). This discussion is consistent with the observation that PVT activates a fronto-parietal network involved in attention, motivation, sensory information integration and motor programming (22) that may have been affected by the ingestion of the noradrenaline reuptake inhibitor. However, we cannot completely rule out that Rebox has influenced other neurotransmitter systems (26) and that they have contributed to the effect of Rebox on central fatigue. Although Rebox is known to have a much higher potency for noradrenaline compared with dopamine and serotonin (26), in brain areas where noradrenergic terminals are in abundance (such as the medial prefrontal cortex), elevated dopamine concentrations occur due to the inhibition of dopamine reuptake by the noradrenaline transporters (23). Thus, dopamine and noradrenaline are intrinsically linked via chemical pathways, in that hydroxylation of the former yields the latter (7).

Paragraph 43 In conclusion, the alteration of the cycling performance due to the ingestion of a noradrenaline reuptake inhibitor was associated with a greater decrease in voluntary activation and PVT performance than in the Pla condition in well-trained male subjects. In contrast, dopamine reuptake inhibition had no additional effect than placebo in temperate conditions. These original findings suggest that noradrenaline, but not dopamine reuptake inhibition, contributes to the development of supraspinal fatigue observed after a prolonged cycling exercise performed in temperate environment. Because corticospinal

excitability was not modified after the cycling exercise, noradrenaline appears to affect more specifically the supraspinal centers located prior to the motor cortex. As a consequence, the output from the motor cortex becomes insufficient to drive the muscles optimally and thereby likely contributes to affect motor performance.

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Figures legends

Figure 1. Experimental protocol. The session began with pre-exercise recordings followed by the cycling exercise and post-exercise recordings at 10, 15, 30 and 45 min after the end of the time trial (see experimental protocol section for further information). *ES,* electrical motor nerve stimulation; *PVT,* psychomotor vigilance task; *TMS*, transcranial magnetic stimulation; *Wmax*, maximal power output.

Figure 2. Change in mean power output during the cycling exercise (A) and in MVC torque after the time trial (B) for placebo (*Pla*), reboxetine (*Rebox*) and methylphenidate (*Mph*) conditions. In A, data are expressed as percentage of the power fixed at the beginning of the time trial (*% initial*). In B, data are expressed as percentage of pre-exercise value (*% control*) at 10 min (*R10*), 30 min (*R30*), and 45 min (*R45*) after the end of the time trial. Values are means ± SEM. Significant difference from pre-exercise value: $*P < 0.05$.

Figure 3. Change in voluntary activation tested by TMS and by supramaximal electrical motor nerve stimulation for placebo (*Pla*), methylphenidate (*Mph*) and reboxetine (*Rebox*) conditions. A and B, superimposed twitch (*SIT*) torques and corresponding EMG responses (*MEP and Mmax*) induced by TMS (A) or electrical stimulation (B) during MVC in the rectus femoris of a representative subject. Traces obtained during pre-exercise recordings (*grey line*) and 10 min after the end of the time trial (*black line*) are superimposed. Arrow indicates the timing of the stimulus. C and D, change in voluntary activation tested by TMS (C) or electrical stimulation (D) before (*Control*), 10 min (*R10*), 30 min (*R30*) and 45 min (*R45*) after the end of the time trial. Values, expressed in percentage of maximum, are means ± SEM. Significant difference from pre-exercise value: $*P < 0.05$.

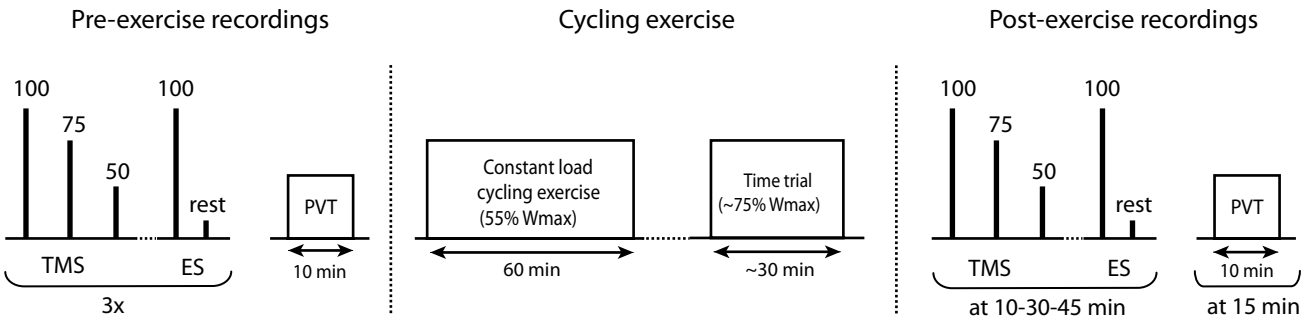
Pre-exercise recordings
Cycling exercise
Post-exercise recordings
100
75
50
100
rest
TMS
ES
3x
PVT
10 min
Constant load
cycling exercise
(55% Wmax)
60 min
Time trial
(~75% Wmax)
~30 min
100
75
50
100
rest
TMS
ES
at 10-30-45 min
PVT
10 min
at 15 min

Figure 1

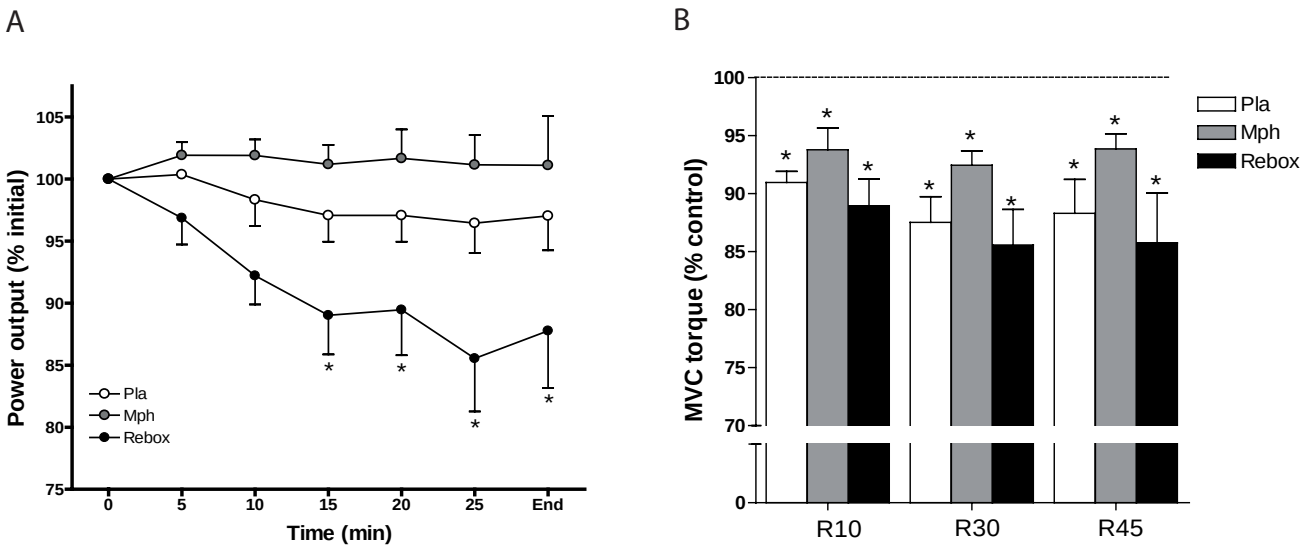
A
B
Power output (% initial)
105
100
95
90
85
80
75
0
5
10
15
20
25
End
Time (min)
Pla
Mph
Rebox
*
MVC torque (% control)
100
95
90
85
80
75
70
0
R10
R30
R45
Pla
Mph
Rebox

Figure 2

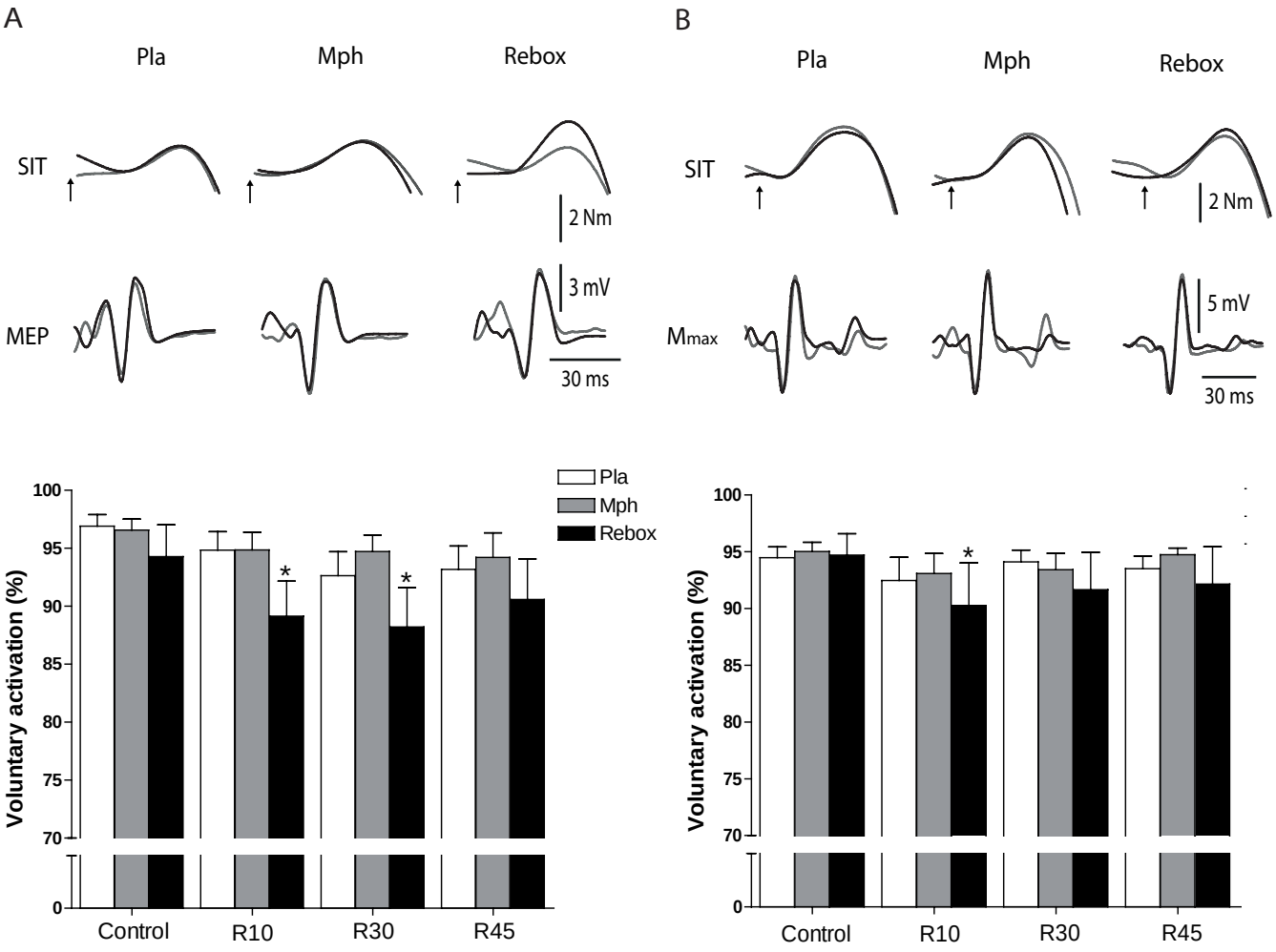
A
Pla
Mph
Rebox
SIT
2 Nm
MEP
3 mV
30 ms
B
Pla
Mph
Rebox
SIT
2 Nm
Mmax
5 mV
30 ms
Pla
Mph
Rebox
Voluntary activation (%)
100
95
90
85
80
75
70
0
Control
R10
R30
R45
*
*
Voluntary activation (%)
100
95
90
85
80
75
70
0
Control
R10
R30
R45
*

Figure 3

Table 1. MEP area, latency, silent period duration, and Mmax area measured at 100% MVC during pre-exercise recordings (*Control*) and 10 min after the end of the time trial (*R10*) in rectus femoris and vastus medialis muscles

	Rectus femoris		**Vastus Medialis**	
	Control	**R10**	**Control**	**R10**
MEP area (% Mmax)				
Pla	62.6 ± 13.9	60.0 ± 12.7	66.0 ± 20.1	68.5 ± 20.7
Rebox	58.4.0 ± 7.9	61.1 ± 11.4	72.2 ± 22.0	66.8 ± 22.4
Mph	64.5 ± 9.5	62.3 ± 13.3	65.6 ± 22.2	70.3 ± 23.7
MEP latency (ms)				
Pla	21.0 ± 1.9	20.7 ± 1.9	22.4 ± 1.4	22.2 ± 1.3
Rebox	21.2 ± 1.8	21.4 ± 1.9	22.7 ± 2.1	23.0 ± 1.8
Mph	20.9 ± 1.3	20.7 ± 1.2	22.4 ± 1.0	22.0 ± 1.0
Silent period (ms)				
Pla	101.3 ± 10.8	100.0 ± 10.1	101.0 ± 7.6	100.5 ± 7.6
Rebox	102.4 ± 8.8	102.9 ± 8.2	101.6 ± 10.5	100.4 ± 10.2
Mph	101.4 ± 11.1	101.1 ± 8.3	101.7 ± 10.6	100.4 ± 7.5
Mmax area (µV.s)				
Pla	60.5 ± 25.9	60.3 ± 23.8	54.4 ± 33.3	55.0 ± 33.1
Rebox	67.9 ± 17.9	64.9 ± 19.8	56.2 ± 28.3	66.7 ± 38.7
Mph	55.7 ± 24.1	54.3 ± 22.5	47.3 ± 26.2	46.5 ± 27.0

Data are means ± SD. No data differ significantly between *Control* and *R10* for the three conditions (*Pla, Rebox* and *Mph*).

Table

Table 2. Peak torque, time-to-peak torque and half-relaxation time of the twitch induced by motor nerve stimulation during pre-exercise recordings (*Control*) and 10 min after the end of the time trial (*R10*)

	Control	**R10**
Peak torque (Nm)		
Pla	46.1 ± 12.0	40.5 ± 8.7*
Rebox	41.9 ± 9.5	34.3 ± 6.9*
Mph	45.0 ± 10.5	37.8 ± 12.2*
Time-to-peak torque (ms)		
Pla	73.0 ± 4.4	68.1 ± 3.0*
Rebox	76.4 ± 8.6	73.8 ± 10.8*
Mph	73.3 ± 5.1	67.4 ± 3.4*
Half-relaxation time (ms)		
Pla	90.3 ± 13.8	75.5 ± 13.7*
Rebox	90.1 ± 14.5	76.9 ± 16.9*
Mph	88.0 ± 16.4	69.0 ± 10.4*

Data are means ± SD. *Significant difference between *Control* and *R10* ($P < 0.05$).

Table 3. PVT reaction time and number of lapses measured during pre-exercise recordings (*Control*) and 15 min after the end of the time trial (*R15*)

	Control	**R15**
Reaction time (ms)		
Pla	314.4 ± 29.6	310.4 ± 33.2
Rebox	309.6 ± 34.1	330.4 ± 36.7*
Mph	309.6 ± 34.4	292.2 ± 30.6*
Number of lapses		
Pla	5.6 ± 5.8	5.1 ± 4.0
Rebox	4.4 ± 4.7	7.1 ± 3.9
Mph	2.9 ± 3.4	2.8 ± 3.0

Data are means ± SD. *Significant difference between *Control* and *R15* ($P < 0.05$).