Acute norepinephrine reuptake inhibition decreases performance in normal and high ambient temperature

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Acute norepinephrine reuptake inhibition decreases performance in normal and high ambient temperature. J Appl Physiol 105: 206–212, 2008. First published May 22, 2008; doi:10.1152/japplphysiol.90509.2008. —Combined inhibition of dopamine (DA)/norepinephrine (NE) reuptake improves exercise performance and increases core temperature in the heat. A recent study demonstrated that this effect may primarily be related to increased DA activity. NE reuptake inhibition (NERI), however, has received little attention in humans, certainly in the heat, where central fatigue appears to be a main factor influencing performance. Therefore the present study examines the effect of NERI (reboxetine) on exercise capacity, thermoregulation, and hormonal response in normal and high temperature. Nine healthy well-trained male cyclists participated in this study. Subjects ingested either placebo (Pla; 2 × 8 mg) or reboxetine (Rebox; 2 × 8 mg). Subjects exercised in temperate (18°C) or warm (30°C) conditions and cycled for 60 min at 55% Wmax immediately followed by a time trial (TT; Pla18/Rebox18; Pla30/Rebox30) to measure exercise performance. Acute NERI decreased power output and consequently exercise performance in temperate (P = 0.018) and warm (P = 0.007) conditions. Resting heart rate was significantly elevated by NERI (18°C: P = 0.02; 30°C: P = 0.018). In Rebox18, heart rate was significantly higher than in the Pla18, while in the heat no effect of the drug treatment was reported during exercise. In Rebox30, all hormone concentrations increased during exercise, except for growth hormone (GH), which was significantly lower during exercise. In Rebox18, prolactin (PRL) concentrations were significantly elevated; GH was significantly higher at rest, but significantly lower during exercise. In conclusion, manipulation of the NE system decreases performance and modifies hormone concentrations, thereby indicating a central NE effect of the drug. These findings confirm results from previous studies that predominantly increased DA activity is important in improving performance.

central fatigue; reboxetine; exercise; heat

Changes in the synthesis and metabolism of central neurotransmitters have been suggested to contribute to the genesis of fatigue during prolonged exercise (17). Piacentini and colleagues (15, 24–26) investigated the effects of a serotonin (5-HT; fluoxetine), a dopamine (DA)/norepinephrine (NE) (DA/NE; bupropion), a NE (reboxetine; Rebox), and a 5-HT/NE (venlafaxine) reuptake inhibitor on performance and hormonal parameters in humans in normal ambient temperature. The authors failed to show any changes in performance, although a trend to a decrease in performance was found after Rebox administration. The authors did detect different hormonal responses, depending on the neurotransmitter system triggered by the reuptake inhibitor. The neuroendocrine response to exercise suggested that the pharmacological manipulations indeed produce a central effect, despite a failure to influence performance.

Capacity to perform prolonged exercise is clearly impaired in high ambient temperatures (23). In the heat, exercise capacity is thought to be primarily limited by thermoregulatory and fluid balance factors (9), but it has been suggested that when body temperature is elevated, the central nervous system (CNS) becomes important in the development of fatigue (22, 37). Recently, Watson et al. (37) examined the effects of a DA/NE reuptake inhibitor (bupropion) in temperate and warm environmental conditions. The major finding was that subjects completed the time trials in the heat 9% faster when the DA/NE systems were influenced, an effect that was not apparent in 18°C. Seven of nine subjects attained body core temperatures (Tcore) exceeding 40°C, implying that DA and/or NE may dampen or override hyperthermia-induced inhibitory signals arising from the CNS to stop exercising, potentially increasing the risk of developing heat illness. Since the increase in central catecholaminergic neurotransmission may, in part, attenuate the loss of performance when exercise is performed in warm environmental temperatures (37), and DA and NE are involved in motor behavior, motivation, and drive (17), it is interesting to elucidate the specific role of DA and NE in this process.

The role of DA has very recently been studied in our laboratory. Methylphenidate (mph; Ritalin), a DA reuptake inhibitor, improved time trial performance in warm but not in normal ambient temperatures (31). The results of this study showed a clear effect of the DA system on performance (16% improvement on a 30-min time trial) that was not apparent in 18°C. Furthermore, the combination of a DA reuptake inhibitor and high ambient temperature increased Tcore at rest and during exercise, with subjects reaching an average Tcore of 40°C or above, while there was only one subject who reached 40°C in the placebo trial. This increased Tcore is caused by the increased heat production, resulting from an increased motiva-
tion and drive to continue, enabling subjects to maintain a higher power output compared with the placebo condition (31). The perceptual response to the increases in T\text{core} and heart rate seems to be dampened, since no differences in ratings of perceived exertion (RPE) and thermal stress were found, a result that confirms the previous study performed with a DA/NE reuptake inhibitor (37). The authors further concluded that the effects exerted by DA reuptake inhibition are even more pronounced than the effects of DA/NE reuptake inhibition (31).

To our knowledge there are no data available in literature of studies that looked into the specific role NE neurotransmission plays in performance and thermoregulation during prolonged exercise in high ambient temperature. NE reuptake inhibition has been investigated during exercise in thermoneutral environments, using Rebox (25). Rebox is widely prescribed as an antidepressant (35); it occupies the NE transporter (NET; 14), thereby increasing the concentration of NE in the synaptic cleft (14), and has little or no affinity for the DA and 5-HT uptake sites or for muscarinic H\text{2}-histaminergic or adrenergic receptors (2). Piacentini and colleagues (25) found that subjects were slower after NE reuptake inhibition and that the hormonal alterations observed indicated a central effect of the drug due to an increase in NE extracellular concentrations (25). A study in guinea pigs has shown hypothermic properties of NE when microdialyzed in the preoptic area (28). This effect is mediated by α\text{2}-adrenergic receptors (29). Similar results were found in rats after injection of NE in the anterior hypothalamus (8) and in cats after NE application to the thermosensitive zone of the hypothalamus (21). Myers et al. (21) suggested that NE is involved in heat-dissipation mechanisms, thereby evoking hypothermic effects. On the other hand, local application of NE in the preoptic area and anterior hypothalamus (PO/AH) and acute injection of cirazoline (a NE receptor agonist) increased body temperature (5, 7). These contradictory findings show that the exact role of NE in thermoregulation is not yet understood.

The effect of NE reuptake inhibition on exercise performance and thermoregulation has received little attention in humans, certainly in the heat. However, it appears that the influence of manipulating with the DA and NE systems becomes important specifically during exercise in the heat, where central fatigue appears to be a main factor influencing performance. Therefore, the purpose of the present study is to examine the effect of NE reuptake inhibition on exercise capacity, thermoregulation, and the hormonal response to exercise. We hypothesize that acute administration of the NE reuptake inhibitor Rebox will decrease exercise performance, exert hypothermic effects, and influence hormone concentrations during exercise in temperate and hot environmental conditions.

METHODS

Subjects. Nine healthy men (age 23 ± 4 yr; height 1.76 ± 0.08 m; mass 73 ± 9 kg; W\text{max} 335 ± 30 W) participated in this investigation. All subjects were trained cyclists or triathletes but were not accustomed to exercise in a warm environment at the time of the study. Before the start of the study, all volunteers received written information regarding the nature and purpose of the experimental protocol. Following an opportunity to ask any questions, a written statement of consent was signed. The protocol employed was approved by the Research Council of the Vrije Universiteit Brussel (Brussels, Belgium).

Experimental protocol. The experimental protocol used in this study is identical to the protocol used previously (31, 37); therefore we will address it briefly. All subjects completed a preliminary maximal exercise test, a familiarization trial, and four experimental trials. The preliminary trial consisted of continuous incremental cycle exercise to volitional exhaustion and was used to determine the power output required to elicit 55% and 75% of maximal workload. A familiarization trial was undertaken to ensure the subjects were accustomed to the procedures employed during the investigation and to minimize any potential learning or anxiety effects. This trial was performed in temperate environmental conditions and was identical to the experimental trials in all respects. Experimental trials were undertaken in either temperate (18°C) or warm (30°C) conditions [trials are referred to as placebo at 18°C (Pla18), placebo at 30°C (Pla30), reboxetine at 18°C (Rebox18), and Reboxetine at 30°C (Rebox30)], with relative humidity maintained between 50% and 60% in both conditions. All subjects had to complete all experimental trials, which were separated by at least 7 days to minimize the development of heat acclimation and to ensure drug washout. Subjects were instructed to record dietary intake and physical activity during the 2 days before the first trial and to replicate this in the 2 days before the subsequent experimental trials. No exercise, caffeine, or alcohol consumption was permitted in the 24 h before each trial.

Subjects entered the laboratory in the morning ~90 min after consuming a standardized breakfast that included 500 ml of plain water. Nude postvoid body mass was measured, and an indwelling venous cannula was introduced into a superficial forearm vein to enable repeated blood sampling at rest and during exercise. Subjects inserted a rectal thermometer (Gram LT-8A, Saitama, Japan) 10 cm beyond the anal sphincter for the measurement of T\text{core}. Surface skin temperature probes (Gram LT-8A) were attached to four sites (chest, upper arm, thigh, and calf) to enable the determination of weighted mean skin temperature (30), and a heart rate telemetry band (Polar Accurex plus, Kempele, Finland) was positioned. Subjects were dressed in only cycling shorts, socks, and shoes for all trials.

Subjects then entered a climatic chamber maintained at the appropriate environmental conditions and rested in a seated position for 15 min. During this period temperatures and heart rate were recorded at 5-min intervals and a resting venous blood sample was drawn; blood pressure was measured immediately before the start of exercise. The exercise protocol consisted of 60-min constant-load exercise at a workload corresponding to 55% W\text{max} followed by a time trial (TT) to measure exercise performance. There was a 1- to 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% W\text{max} as quickly as possible (13). Subjects began the TT at a workload corresponding to 75% W\text{max} 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 subjects an indication of their 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.

Core and skin temperatures and heart rate were recorded at 5-min intervals during exercise. Ratings of perceived exertion (RPE; 3) and thermal stress (assessed using a 21-point scale ranging from unbearable cold to unbearable heat) were assessed every 15 min during the initial 60 min and at 10-min intervals during the TT. Venous blood samples were drawn and blood pressure measured after 60 min of constant-load exercise and at the end of the TT. Following the completion of the TT, subjects returned to a seated position where recovery was monitored for 15 min and a further blood sample was obtained, together with a measurement of the blood pressure. The
probes and cannula were then removed, and nude body mass was then remeasured to allow the estimation of body weight losses.

**Drugs.** Subjects ingested 8 mg Rebox or a placebo (Pla; lactose) on the night before the experiment and 8 mg on the morning of the experiment. The treatment was randomized and administered in double-blind crossover manner. Rebox and Pla capsules were prepared by an independent pharmacy to appear indistinguishable with regard to dimensions, weight, and color.

**Blood collection, analysis, and blood pressure.** Venous blood samples were drawn directly into precooled vacutainer tubes (BD Vacutainer, Plymouth, UK). Ten-milliliter samples were collected into plain tubes and left to clot for 1 h at room temperature before centrifugation. The resulting serum was stored at −20°C for the determination of prolactin (PRL; Roche Diagnostics, Mannheim, Germany), cortisol (Diasorin), and growth hormone (GH; Pharmacia & Upjohn Diagnostics, Uppsala, Sweden). Samples for plasma adrenocorticotropic hormone (ACTH) were collected into 4.5-ml tubes containing K3EDTA. An additional 7.5 ml was added to lithium heparin. A 0.5-ml aliquot of whole blood was extracted and used for the determination of hematocrit. To measure the blood lactate concentration, blood samples (20 µl) were drawn from an arterialized ear lobe. Lactate concentration was determined enzymatically (EKF; BIOSEN 5030, Magdeburg, Germany). Blood pressure was measured with an automatic unit for blood pressure measurements at the upper arm (Bosos, Bosch, and Sohn).

**Statistical analysis.** Data are presented as means ± SD. The one-sample Kolmogorov-Smirnov test showed that all outcome variables had a normal distribution. To evaluate differences in TT performance and power output, a paired t-test was employed. In the present study we compared within-temperature conditions and not between-temperature conditions. Data collected over time were analyzed using two-factor (drug × time) ANOVA with repeated measures. Statistical significance was accepted at \( P < 0.05 \). Paired t-tests were used to identify pairwise differences.

**RESULTS**

All subjects completed all the experimental trials. Over half of the subjects at some point complained of unexplainable cold feelings in both normal and high ambient temperature, only after Rebox administration. Subjects took longer to complete the predetermined amount of work in the Rebox trials compared with the corresponding Pla trials. Acute Rebox supplementation prolonged exercise time in temperate \( (P = 0.018) \) and in warm \( (P = 0.007) \) conditions. Subjects finished 10 and 20% slower in the respective Rebox18 and Rebox30 TT compared with the Pla (Pla18: 29 min 54 s ± 1 min 18 s; Rebox18: 32 min 54 s ± 3 min 42 s; Pla30: 40 min 36 s ± 6 min 24 s; Rebox30: 48 min 36 s ± 10 min 54 s; Fig. 1).

As the TT required the completion of a predetermined amount of work, the time taken to complete the protocol was directly related to the power output maintained throughout this period. In both the Pla18 and Pla30 time trials, the power output was significantly higher than in the respective Rebox time trials (Pla18: 250.7 ± 30.9 W; Rebox18: 230.5 ± 39.7 W; \( P = 0.009 \); Pla30: 189.6 ± 49.2 W; Rebox30: 166.3 ± 51.9 W; \( P = 0.007 \)).

\( T_{core} \) increased during exercise in all trials. \( T_{core} \) was not significantly influenced at rest or during exercise by the drug administration in both temperate and warm environmental temperature. Only during the recovery phase after Rebox18 was \( T_{core} \) significantly higher than in the Pla18 recovery (after 5 min: Pla18 37.5 ± 1.4°C, Rebox18 37.9 ± 1.2°C, \( P = 0.029 \); after 10 min: Pla30 37.2 ± 1.3°C, Rebox30 37.6 ± 1.1°C, \( P = 0.035 \); Fig. 2). Skin temperature increased during exercise in all conditions, reaching a plateau after 15 min in Pla30 and Rebox30 and after 30 min in Pla18 and Rebox18. No differences in weighted mean skin temperature were obvious between Pla and NE reuptake inhibition.

Resting heart rate was significantly elevated by NE reuptake inhibition (18°C: \( P = 0.02 \); 30°C: \( P = 0.018 \); Fig. 3, A and B). In Rebox18 heart rate remained elevated above the values of the Pla trial during the 60-min fixed-intensity exercise \( (P < 0.028) \) and after 10 and 15 min of recovery \( (P < 0.036) \); Fig. 3, A and B). In the heat, no effect of the drug treatment was reported during or after exercise.
RPE was similar between Pla and Rebox treatment in temperate and warm conditions. The subjects' ratings of thermal stress were influenced by the drug treatment. Although no statistical difference was found, there was a clear trend toward lower thermal stress scores in both temperate and warm conditions after Rebox administration (Fig. 4, A and B). At the end of the TT ($P = 0.021$; after recovery: $P = 0.029$; Fig. 5C). Serum PRL was influenced by Rebox in both temperate and warm conditions (Fig. 5D). In normal temperature PRL concentrations were higher at the end of the TT and after the 15-min recovery period with Rebox administration ($P = 0.044$ and $P = 0.031$, respectively). In the heat, Rebox increased PRL concentration after 60 min of exercise ($P = 0.02$).

**DISCUSSION**

The present study investigated the effects of acute oral administration of a NE reuptake inhibitor (Rebox) on exercise performance, thermoregulation, and hormonal responses to prolonged exercise in both temperate and warm environments.

In contrast to previous studies that used the same protocol as the present study to evaluate the effects of a DA/NE reuptake inhibitor (37) and a DA reuptake inhibitor (31), a NE reuptake inhibitor decreased performance in both normal (10% decrease) and high (20% decrease) ambient temperature. This performance decrement is a confirmation of the trend of a decrease in performance (5.5% or 5 min in a 90-min time trial) already shown by Piacentini et al. (25) with the same drug. The higher dosage used in the present study can explain the more pronounced results.

The present result is in a way unexpected because NE mechanisms are thought to be involved in the control of level of arousal, consciousness, and reward mechanisms in the brain (16, 17, 19), suggesting NE could play a role in the enhancement of performance. On the other hand, it is well established that NE neurons modulate the 5-HT system via excitatory $\alpha_1$-adrenoceptors. In the brain stem, dorsal raphe 5-HT neurons receive ascending NE neuron afferents originating from the locus ceruleus (35). 5-HT has been implicated in central fatigue (17), and there is pharmacological evidence from both animal and human studies that is consistent with the suggestion...
that an increase in central 5-HT neurotransmission is detrimental to the performance of prolonged exercise (18, 33, 38).

The present study followed the exact same protocol as previous work (31, 37). Comparing all the results, it becomes evident that in the heat, the DA neurotransmission is important for improving performance and increasing metabolic heat production to levels that may potentially increase the risk of developing heat illness (31, 37). The inhibition of the reuptake of DA enables subjects to push into the “danger zone,” close to critical core temperatures, without, or with significantly dampened, negative feedback from the CNS (37). The role DA plays in the mesolimbic reward system seems to be important for this reaction and the increase in performance (10, 36).

The negative effect NE exerts on performance is not accompanied by a significant change in $T_{\text{core}}$, although there is a trend for a lower temperature during the trial in the heat after NE reuptake inhibition. This trend is further acknowledged by the subjects’ thermal stress scale scores that indicate that the subjects felt colder after NE reuptake inhibition. Several subjects also complained about cold feelings after NE reuptake inhibition.

Fig. 5. Concentrations of cortisol (A), adrenocorticotropic hormone (ACTH; B), growth hormone (GH; C), and prolactin (D) (mean ± SD). *Significant difference between the Pla trial and the corresponding time point on the Rebox trial ($P < 0.05$).
blockage (overnight or in the morning of the test). Earlier studies already showed a hypothermic effect after NE administration. Quan et al. (28) found this response after acute NE injection in the guinea pig PO/AH; Gisolfi and Christman (8) (rat PO/AH) and Myers et al. (21) (cat PO/AH) also found hypothermic effects. Quan and colleagues (29) reported that this hypothermic response is mediated by α2-noradrenergic receptors in the PO/AH (29), while studies agonizing α1-noradrenergic receptors found rapid rises in Tcore (7, 12). From agonist induced a fall in Tcore that lasted for at least 3 h (7). This hypothermic response is mediated by hypothermic effects. Quan and colleagues (29) reported that (rat PO/AH) and Myers et al. (21) (cat PO/AH) also found administration. Quan et al. (28) found this response after acute NE reuptake inhibition, both in normothermia and in hyperthermia. The fact that acute NE reuptake inhibition has an enhancing effect on PRL concentrations has already been shown by Piacentini et al. (25) and Schüle et al. (32). Higher ACTH concentrations after NE reuptake inhibition have been shown by Piacentini et al. (25) in normothermia, while no effect on cortisol was observed. In the present study we found an increase in both ACTH and cortisol only in the higher environmental temperature. The enhanced NE concentrations in the synaptic cleft stimulate corticotropin-releasing hormone output (11, 32), thereby stimulating ACTH secretion. The hormonal modifications observed in the present study indicate that the drug had a central effect.

In conclusion, acute NE reuptake inhibition resulted in a significant increase in the time to complete a predetermined amount of work in both normal and warm environmental temperature. In contrast to studies that increase DA/NE neurotransmission (37) and DA neurotransmission (31), an inhibition of the reuptake of NE did not induce hyperthermia in healthy subjects. Manipulation of the NE neurotransmitter system rather induced feelings of cold, without any change in the perception of effort. Although we cannot rule out the possible peripheral effects of NE reuptake inhibition, the decrease in performance and the hormonal modifications indicate that NE reuptake inhibition has a central effect.

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