A Catecholamine Precursor Does Not Influence Exercise Performance in Warm Conditions

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ABSTRACT

CORDERY, P., L. J. JAMES, N. PEIRCE, R. J. MAUGHAN, and P. WATSON. A Catecholamine Precursor Does Not Influence Exercise Performance in Warm Conditions. *Med. Sci. Sports Exerc.*, Vol. 48, No. 3, pp. 536–542, 2016. **Purpose**: Acute doses of Sinemet® (L-DOPA combined with carbidopa) previously failed to influence prolonged exercise performance in a temperate environment, but it is not known whether acute doses of L-DOPA timed to reach maximum plasma concentrations (C_{max}) during exercise will improve prolonged cycling performance in warm conditions ($30.2°C \pm 0.2°C$, $50\% \pm 1\%$). **Methods**: Ten physically active men (age, 26 ± 4 yr; height, 1.76 ± 0.08 m; body mass, 76.3 ± 10.6 kg; $\dot{V}O_{2peak}$, 57 ± 8 mL·kg^{-1}·min^{-1}) were recruited for this study. Participants cycled for 1 h at 60% $\dot{V}O_{2peak}$ followed by a 30-min exercise test, during which they were instructed to complete as much work as possible. Heart rate, skin and core temperatures, as well as RPE and thermal stress were recorded throughout the exercise, and blood samples were collected at rest, at 15-min intervals during the first hour of exercise, and at the end of the exercise test. Finger tapping tests at the beginning and end of the exercise were conducted to examine fine motor control. **Results**: There was no significant difference in the work done on the placebo (314 ± 43 kJ) and L-DOPA trials (326 ± 48 kJ, $P = 0.276$). Prolactin concentrations were increased at the end of the exercise in all trials ($P < 0.001$), but this response was attenuated at the end of the exercise for the L-DOPA trial (11.4 ± 5.5 ng·mL^{-1}) and placebo trials (20.8 ± 3.3 ng·mL^{-1}, $P = 0.003$). No differences between trials were found for any other measure. **Conclusions**: The results suggest that increasing central catecholamine availability inhibits the normal prolactin response to exercise in the heat but does not alter performance, thermoregulation, or sympathetic outflow. **Key Words**: DOPAMINE, NOREPINEPHRINE, DOPING, CENTRAL FATIGUE, SINEMET

Endurance exercise capacity is reduced by increasing ambient temperature (13). Hyperthermia has been found to impair maximal muscle activation, alter brain activity, and increase perceived exertion, and these physiological stressors result in the eventual fatigue via mechanisms residing within the CNS (24). Although the cerebral mechanisms for the onset of fatigue are not completely understood, many studies have attempted to manipulate fatigue by altering the CNS function. Pharmacological inhibition of catecholamine reuptake has been consistently found to improve performance during prolonged exercise in warm conditions (28,30,40). However, attempts to manipulate central fatigue using precursors for catecholamine synthesis have produced conflicting results. Several studies of prolonged military operational drills found that supplementation with L-tyrosine reduced fatigue and stress while improving cognitive and motor performance in soldiers (25,32,34), but laboratory exercise studies in humans conducted under better-controlled conditions have found no benefit of L-tyrosine supplementation (4,35). The apparent disparity in effectiveness might be explained by the nature, magnitude, and duration of physiological and psychological stress endured by soldiers compared with that typically experienced by volunteers in laboratory studies. Although a recent report suggests that acute tyrosine administration before exercise enhances exercise capacity in warm conditions (36), a subsequent study by the same authors found no effect on performance (37). In addition, an independent study using a similar experimental protocol reported no difference in exercise time to exhaustion (39).

These equivocal findings perhaps suggest that L-tyrosine does not sufficiently increase catecholamine synthesis under these conditions. Tyrosine hydroxylase is the rate-limiting enzyme in the synthesis of catecholamines and, under normal physiological conditions, is close to saturation (11). Consequently, administration of L-DOPA, thus bypassing this step, is markedly more effective than L-tyrosine at increasing catecholamine synthesis. L-DOPA has been used to treat motor control disorders in Parkinson disease for over 40 yr and is considered the "gold standard" treatment today (22). The therapeutic action of L-DOPA on motor function is primarily due to increased dopamine synthesis. However, L-DOPA also increases noradrenaline synthesis which contributes to its effects in Parkinson disease (7). Clinically, L-DOPA is administered with an amino acid decarboxylase inhibitor that cannot readily cross the blood–brain barrier; this prevents decarboxylation of L-DOPA in the periphery, thereby

reducing associated gastrointestinal distress and increasing the delivery of L-DOPA to the brain.

To date, only one study has investigated the effects of L-DOPA on prolonged exercise performance (18). This study was conducted in a temperate environment and reported no effect on exercise performance. This study used Sinemet®, a combination drug containing L-DOPA and carbidopa (amino acid decarboxylase inhibitor) in a ratio of 4/1. Absorption of Instant Sinemet® begins about 30 min after ingestion, the C_{max} of L-DOPA occurs roughly 1 h after ingestion, and the half-life is short, being approximately 2 h (5). In the study by Meeusen et al. (18), the dosing protocol is described as 4 mg·kg^{-1} Sinemet® and was taken 24 h before exercise and on the morning of the trial. The study imposed a standardized breakfast before exercise, which would affect L-DOPA pharmacokinetics, severely reducing circulating L-DOPA concentrations (5). Therefore, the dosing protocol used does not seem ideal with respect to L-DOPA pharmacokinetics. Nonetheless, peripheral catecholamine concentrations were increased, as was the circulating growth hormone, suggesting that the expected central effect of the treatment was apparent. This study found no effect on exercise performance, but the effects of central catecholaminergic manipulation do appear to be more pronounced during exercise undertaken in warm conditions. Therefore, the aim of the present study was to determine the effects of a dosing protocol designed to provide peak L-DOPA concentrations during a performance test involving prolonged exercise performed in a warm environment. It was hypothesized that an acute dose of L-DOPA would increase exercise capacity during prolonged exercise in a warm environment.

METHODS

With approval from the local ethical advisory committee, 10 physically active men (mean ± SD: age, 26 ± 4 yr; height, 1.76 ± 0.08 m; body mass, 76.3 ± 10.6 kg; $\dot{V}O_{2peak}$, 57 ± 8 mL·kg^{-1}·min^{-1}) were recruited to participate in this study. All participants took part in regular endurance exercise but were not accustomed to exercise in a warm environment at the time of the study. Participants were given a written description of the purpose and design of the study, including manufacturer information about the drug treatment (Sinemet®; Merck Sharp & Dohme Ltd Hertfordshire, UK). Those with a history of cardiovascular, metabolic, musculoskeletal, and psychological disorders were to be excluded from the study. Thereafter, if participants confirmed their interest and eligibility, a statement of informed consent was signed.

Participants visited the laboratory five times in total. The first visit consisted of incremental cycle exercise to volitional exhaustion on an electrically braked cycle ergometer (Lode Corival, Groningen, Holland) at 21°C ± 0.1°C, 50% ± 1% relative humidity, which was used to determine $\dot{V}O_{2peak}$ and the power outputs required to elicit 60% and 75% $\dot{V}O_{2peak}$. Subsequently, participants returned for an initial familiarization trial; this followed the same format as the experimental trials (minus any treatment) and was undertaken to ensure that subjects were accustomed to the procedures used during the investigation, to minimize any learning effect, and to ensure that the volunteers were capable of consistently producing a maximal effort during the time trial. This was followed by a single-blind, placebo-controlled trial, which served both as an additional comparison against experimental trials and as second familiarization. The experimental trials that followed were arranged in a randomized double-blind, placebo-controlled crossover design. All trials were separated by at least 7 d to minimize the development of heat acclimation. In the 48 h before experimental trials, participants were asked to avoid heavy exercise and alcohol as well as to replicate the diet and activity recorded in a diary before the first trial.

Upon waking on the day of testing, after an overnight fast, participants ingested Sinemet® (100 mg of L-DOPA/25 mg of carbidopa) or the placebo (glucose) with water. After 1.5 h, participants consumed a standardized breakfast, consisting of two small cereal bars and 500 mL of orange juice; the breakfast contained no protein to prevent competition at the blood–brain barrier large neutral amino acid transporter. They consumed their second dose of Sinemet® or placebo 1.5 h later. Participants were asked to consume 500 mL of water spread over the following 2.5 h, after which they arrived at the laboratory for testing. This dosing protocol was intended to augment central catecholamine stores and achieve peak blood concentrations during exercise while avoiding nausea that is relatively common with administration of L-DOPA. Capsules were prepared by an independent researcher not directly involved in the experimental trials and were identical in weight and color. Upon arrival, participants were asked to void their bowels and bladder before nude body mass was recorded to the nearest 10 g (Adam AFW-120K, Milton Keynes, UK). Subjects inserted a rectal thermistor (Grant Squirrel SQ800, Cambridgeshire, UK) 10 cm beyond the anal sphincter for measurement of core temperature. A heart rate telemetry band (Polar Beat, Kempele, Finland) was positioned, and surface skin temperature probes (Grant Squirrel SQ800) were attached to four sites (chest, upper arm, thigh, and calf) to enable the calculation of weighted mean skin temperature (27). Subjects then rested in a seated position for 15 min in a comfortable environment (22°C–23°C) with one hand immersed in warm water (42°C) for 10 min. A 21-gauge cannula was introduced into a superficial vein of the prewarmed forearm to enable repeated blood sampling, with the cannula flushed with a small volume of heparinized saline after each blood sample. Because of the short time needed to reach C_{max} and the short half-life of L-DOPA, participants were asked to consume a final dose of Sinemet® or placebo immediately before beginning exercise; the total dose provided during the L-DOPA trial was 300 mg of L-DOPA and 75 mg of carbidopa, which is the minimum recommended daily dose initially administered in the management of Parkinson disease.

The experimental protocol is illustrated in Figure 1. After the collection of baseline measurements during the rest
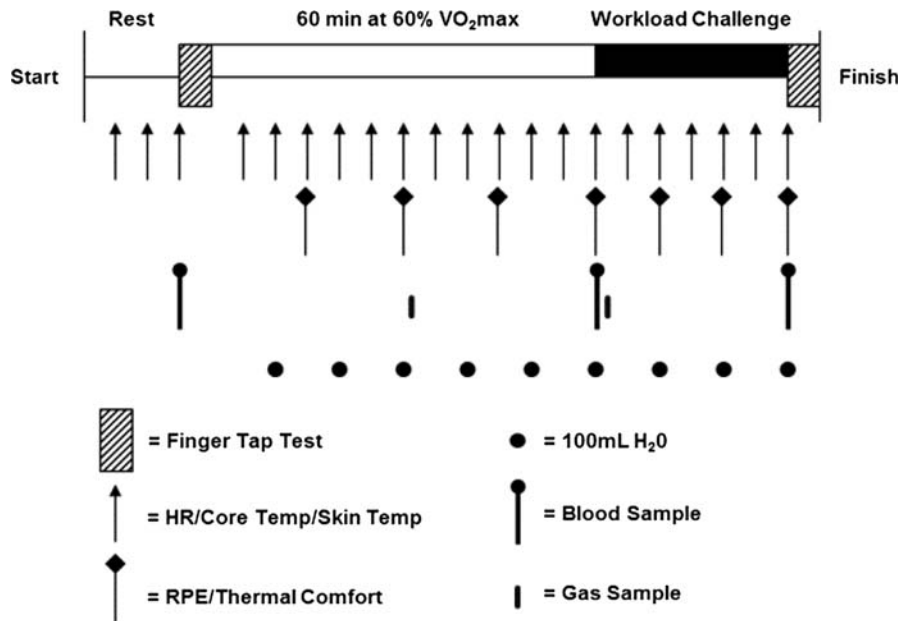
FIGURE 1—Schematic of experimental trials.

period, participants entered the climatic chamber maintained at $30.2°C \pm 0.2°C$, $50\% \pm 1\%$ relative humidity. There was no direct fan cooling, but there was continuous air movement of 1 m·s^{-1} within the environmental chamber. Before and after exercise, participants performed two finger-tapping tests with each hand to determine any changes in fine motor control (33). Because of the short half-life of L-DOPA, extensive cognitive function testing was omitted. Participants were asked to cycle at a work rate corresponding to 60% $\dot{V}O_{2peak}$ for 60 min, followed by a 30-min exercise test, in which participants were asked to complete as much work as possible. Initial power output during the exercise test corresponded to 75% $\dot{V}O_{2peak}$; thereafter, participants were free to manipulate the power output to complete as much work as they felt possible. Standardized verbal encouragement was provided by the experimenter to help ensure a maximal effort. Feedback provided to the participants during the time trial was limited to the time elapsed (power output, cadence, heart rate, etc. were hidden).

During exercise, participants were given 100 mL of water to drink every 10 min. Throughout the trial, heart rate and core and skin temperatures were measured every 5 min. During the 15-min rest period, subjective thermal stress was measured (14). During the 1-h fixed power output period, subjective thermal stress and RPE were measured every 15 min (2). Expired gas samples were also collected at 30 min and at the end of the steady-state exercise period. Subjective thermal stress and RPE were measured every 10 min. After completion of the exercise test and the collection of the final blood sample, participants left the climatic chamber. Skin thermistors were removed, and participants removed the heart rate telemetry band and rectal thermistor in privacy before toweling off, and nude body mass was recorded behind a screen.

This was corrected for the volume of water consumed to allow for the estimation of sweat loss.

During the experimental trials, three 6-mL blood samples were drawn. Samples were collected at rest, at the end of the steady-state exercise period, and upon completion of the time trial. Blood samples were drawn using dry syringes and immediately dispensed into 1- and 2.5-mL tubes containing potassium EDTA (1.5 mg·mL^{-1}) and the remaining whole blood into a plain tube. The 2.5-mL EDTA tubes were kept on ice. The 1-mL EDTA blood samples were used to analyze hemoglobin and hematocrit via the cyanmethemoglobin and microcentrifuge methods, respectively, allowing for estimation of percentage changes in blood, plasma, and red cell volumes relative to the first resting sample (9). Two aliquots of 100 μL of whole blood were pipetted from the 1-mL EDTA tube into microcentrifuge tubes containing 1 mL of 0.3 M perchloric acid kept on ice for measurement of blood glucose using the Randox glucose oxidase phenol 4-aminoantipyrine peroxidase method (Randox Laboratories Ltd, Crumlin, UK). The 2.5-mL EDTA and plain tubes were centrifuged to obtain the plasma and serum, respectively, which were stored at $-80°C$ for hormone analysis. Plasma cortisol and serum prolactin were measured using enzyme-linked immunosorbent assays kits (DRG; International Inc., Springfield, NJ).

Sample size was determined with the statistical software package G*Power 3.0.10. To achieve a power of 0.80 with $\alpha = 0.05$ and a predicted effect size (dz) of 1.00 based on performance data from previous studies (28–30,40), it was determined that a sample size of 10 was required. Data are presented as mean $\pm$ SD. The Shapiro–Wilk test was used to verify that the outcome variables had a normal distribution. Exercise performance data were examined using one-way
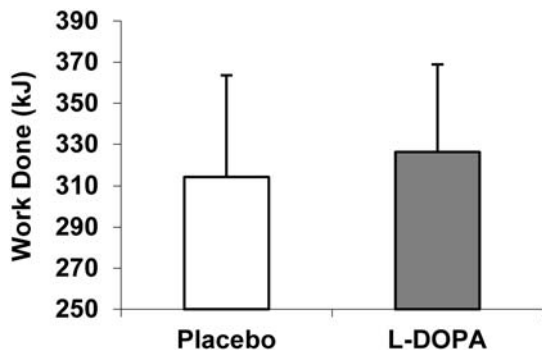
FIGURE 2—Mean total work completed during the exercise test. No significant difference was found between double-blind placebo (314 ± 43 kJ) and L-DOPA trials (326 ± 48 kJ, P = 0.276).

repeated-measures ANOVA and paired-sample *t*-tests. For the finger tapping data, three-way (time-by-trial-by-hand) repeated-measures ANOVA was performed. To identify differences in data collected at multiple time points throughout each trial, two-way (time-by-trial) repeated-measures ANOVA was performed. Where a significant interaction was apparent, pairwise differences were evaluated using the Bonferroni *post hoc* procedure where appropriate. Statistical significance was accepted at $P < 0.05$.

RESULTS

No order effect was observed in the exercise test performance data ($P = 0.553$). Additionally, no significant difference was found in the total work completed between the single-blind (317 ± 49 kJ) and the double-blind placebo trials (314 ± 43 kJ, $P = 0.797$), demonstrating good repeatability of the performance test. No significant difference was found between double-blind placebo (314 ± 43 kJ) and L-DOPA trials (326 ± 48 kJ, $P = 0.276$; Fig. 2). The individual percent changes in performance compared with the placebo are depicted in Figure 3.

Core temperature rose throughout the exercise, but there was no significant difference between trials ($P = 0.863$, Fig. 4A). Weighted mean skin temperature rose rapidly in the first 15 min of exercise after which it plateaued until the end of the exercise (Fig. 4B); this response was not influenced by
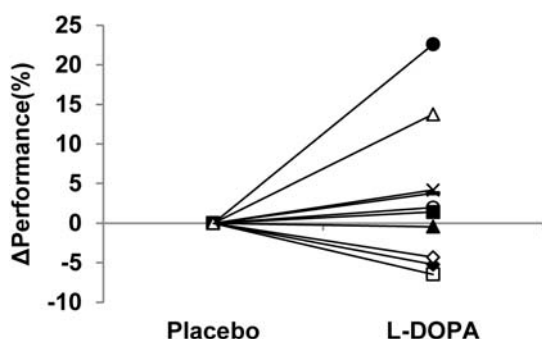

FIGURE 3—Individual percentage change in performance during the L-DOPA trial compared with the placebo.

the treatment administered ($P = 0.930$). Similarly, heart rate rose from the onset of the exercise, then increased gradually throughout the remainder of the steady-state period. Heart rate increased sharply again after the start of the exercise test and continued to rise slowly until the end (Fig. 4C). Again, no significant differences were observed between trials ($P = 0.093$). No differences were observed for sweat loss between placebo and L-DOPA trials (1.87 ± 0.32 vs 1.91 ± 0.31 kg, $P = 0.303$). There was a significant increase in $\dot{V}O_2$ throughout the bout of the steady-state exercise ($P = 0.018$), but there, the treatment did not influence this response (61% ± 5% vs 62% ± 5% $\dot{V}O_{2peak}$, $P = 0.506$).

RPE increased throughout the exercise, but L-DOPA administration did not alter this response compared with the placebo ($P = 0.853$, Fig. 5). Ratings of thermal stress rose rapidly in the first 15 min of exercise but remained stable during the remainder of the steady-state exercise period. During the exercise test, thermal stress ratings rose more rapidly. No significant differences were observed between trials ($P = 0.682$).
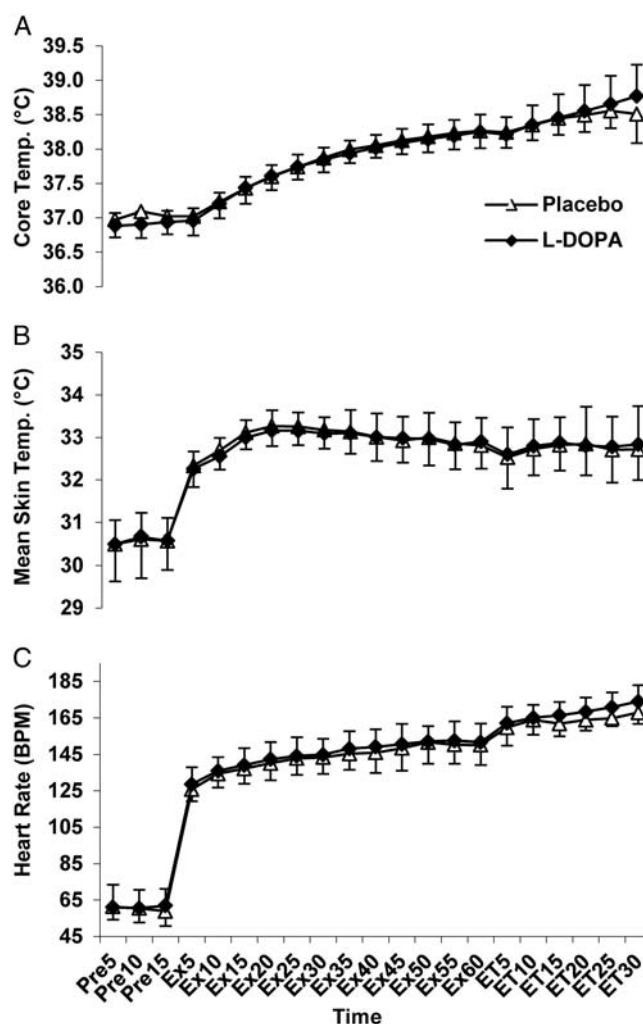

FIGURE 4—Mean core temperature (A), weighted skin temperature (B), and heart rate (C) throughout the experimental trials. No differences between trials were observed in these measurements.
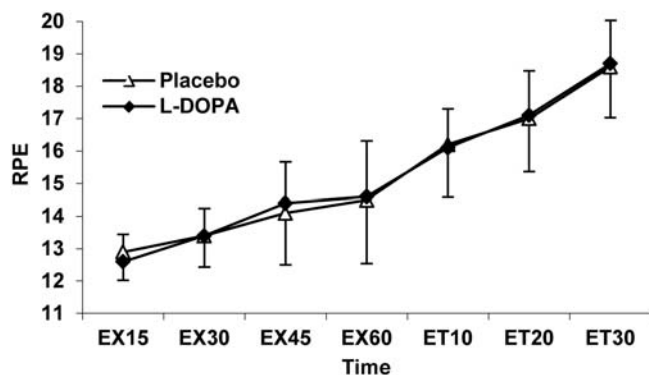
FIGURE 5—Mean RPE throughout the experimental trials. No differences between trials were observed.

Prolactin concentrations were significantly elevated at the end of the exercise in both trials ($P < 0.001$, Fig. 6A), but this response was significantly attenuated during the L-DOPA trial (11.4 ± 5.5 ng·mL^{-1}), compared with the placebo (20.8 ± 3.3 ng·mL^{-1}, $P = 0.003$). Cortisol concentrations were significantly increased at the end of the exercise in both trials ($P = 0.001$, Fig. 6B), but there was no significant difference apparent between trials ($P = 0.294$). Cortisol concentrations were not significantly different between placebo and L-DOPA trials at rest (97.4 ± 27.1 vs 100.8 ± 45.4 ng·mL^{-1}) or at the end of the exercise test (232.2 ± 80.7 vs 246.8 ± 46.1 ng·mL^{-1}).

Finger tapping performance increased after exercise in both trials ($P = 0.023$), but no significant difference was observed for finger tapping performance between trials, hands, hand × time, hand × trial, or hand × time × trial ($P > 0.05$).

DISCUSSION

The results of the present study suggest that augmenting the availability of a catecholamine precursor within the CNS has no effect on performance, thermoregulation, or heart rate during a prolonged exercise test undertaken in a warm environment. Despite clear methodological differences, these findings are broadly in agreement with those of Meeusen et al. (18). Inhibition of the prolactin response was evident in the L-DOPA trial, which is a common effect of L-DOPA treatment that has been well documented (1). This indicates that the dosing protocol produced the desired response of inducing an elevation of brain catecholamine synthesis and release. In the present study, a finger tapping test, which is often used as a diagnostic test for Parkinson disease, was performed to examine possible fine motor control effects. An increase in finger tapping performance of the dominant hand was observed after exercise, but no treatment effect was observed. This may represent a within-trial learning effect due to the participants' desire to improve upon their initial scores, which were displayed on the laptop screen. Improvements observed in finger tapping performance during treatment of Parkinson disease with L-DOPA are a result of temporarily restoring disease-related decrements in striatal dopamine, which should be at optimal levels in healthy

individuals. The lack of effect on heart rate, core temperature, and skin temperature throughout the exercise in the heat are in general agreement with the findings of several previous central catecholamine manipulation studies (28,30,40).

It is possible that these distinct pharmacological interventions have different effects on the central fatigue mechanisms in operation during prolonged exercise in warm conditions. For example, dopamine release appears to be affected by two distinct, but related, pools of presynaptic dopamine: the cytosolic, or alpha-methyl-*para*-tyrosine–sensitive, dopamine pool and the vesicular, or reserpine-sensitive, dopamine pool. Phasic dopamine signaling may be dependent on synaptic vesicle exocytosis, whereas tonic dopamine signaling may be dependent on nonvesicular efflux (21). The L-DOPA–induced increase in dopamine release has been found to be reliant on the reserpine-sensitive pool, via synaptic vesicle exocytosis and phasic signaling (27). These two pools of dopamine appear to play a role in distinguishing drug actions and individual variability in sensitivity and susceptibility to particular drug effects (38). A study using positron emission tomography found that an acute dose of 100 mg of L-DOPA/25 mg of carbidopa (the dose used in the present study) did not increase the resting extracellular dopamine concentration in human striatum at rest in healthy elderly subjects, but did during mental task performance during which phasic activity increases (12). The inhibited prolactin response observed in the present study may be a result of increased phasic dopamine signaling in the hypothalamus.
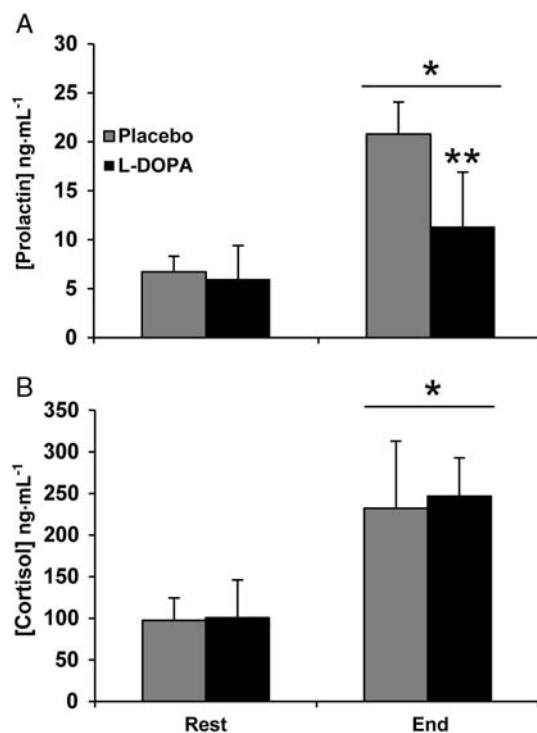

FIGURE 6—A. Mean serum prolactin concentrations before and after exercise in the experimental trials. *Significant difference from rest, $P < 0.001$. **Significant difference between trials, $P = 0.024$. B. Mean plasma cortisol concentrations before and after exercise in experimental trials. *Significant difference from rest, $P = 0.001$.

Tuberoinfundibular dopaminergic neurons in the hypothalamus exert a biphasic control on prolactin secretion from the pituitary via changes in tonic versus phasic firing modes. Prolactin feedback at these neurons shifts to phasic firing to increase dopaminergic inhibition of prolactin secretion (16), whereas the thyrotropin-stimulating hormone shifts these neurons to tonic firing and potentiates the prolactin response (17). This distinction in the effects on tonic and phasic dopamine release may explain, in part, why catecholamine precursors less consistently affect exercise performance than do reuptake inhibitors. Recent evidence suggests that dopamine reuptake inhibition in the nucleus accumbens increases tonic stimulation of low-affinity postsynaptic receptors and results in desensitization to phasic dopamine signals (10). Tonic dopamine signals in the nucleus accumbens modulate baseline extracellular dopamine and set an "average reward" of current behavior, which appears to modulate the response to reward cues (23). Low baseline dopamine levels in the nucleus accumbens shell are associated with decreased motivation and food-seeking behavior, whereas other reward-seeking behavior remains intact in rats (31). Increased tonic baseline dopamine level resulting from reuptake inhibition may result in increased motivation or an enhanced "average reward" of exercise and thereby improve performance by allowing maintained power output. Differing effects on noradrenergic signaling may also contribute to the disparity in performance effects seen between precursors and reuptake inhibitors. L-DOPA administration increases norepinephrine synthesis in the brain (3) and is actively taken up by neurons in the locus coeruleus (19). However, L-DOPA does not affect locus coeruleus activity, whereas concomitant administration potentiates the effects of the norepinephrine reuptake inhibitor reboxetine at the locus coeruleus (20), indicating that augmented neurotransmitter synthesis alone is insufficient to alter activity at the locus coeruleus. In contrast, methylphenidate (8) and bupropion (6) both directly affect locus coeruleus electrophysiology.

In agreement with the present study, the majority of laboratory studies on the effects of an acute dose of a catecholamine precursor have found no benefit to exercise performance (4,18,35,37,39). However, some improvements in performance have been observed for L-tyrosine supplementation during long duration military drills (25,32,34). This may be due to the more stressful nature and longer duration of these studies. During prolonged stress where catecholamines may become depleted because of stress and impair performance, supplementation of precursors, such as tyrosine, may improve performance (15). Although high core temperatures were achieved in the present study, perhaps in more severe thermal stress, L-DOPA may help prevent catecholamine depletion and improve performance.

In summary, provision of L-DOPA results in increased central catecholamine synthesis, but this does not appear to alter prolonged exercise performance in warm conditions. Despite effects on cognitive function and motor cortex plasticity seen in other studies, neither exercise performance nor finger tapping performance was affected by acute augmentation of central L-DOPA availability. Although the provision of catecholamine precursors appears to produce changes in cognitive function and alters striatal phasic dopamine signaling in humans, they do not appear to consistently affect exercise performance. This may suggest that in the majority of healthy individuals, catecholamine synthesis and striatal dopamine are not the determining factors in performance during prolonged exercise in warm conditions. This may change as the duration or stress increase to sufficiently deplete catecholamine stores. In contrast, the relatively consistent increases in exercise capacity seen with catecholamine reuptake inhibitors suggest a particular target and mode of action within the CNS rather than changes in catecholamine metabolism *per se*. Because L-DOPA appears to preferentially increase dopamine quantal release during phasic signaling, it may be that the alteration in tonic catecholaminergic neurotransmission by reuptake inhibitors is more important to improve performance during prolonged exercise in warm conditions.

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