

## ORIGINAL ARTICLE

# Hormonal responses during prolonged exercise are influenced by a selective DA/NA reuptake inhibitor

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**Objective:** A decrease in dopamine activity is thought to lead to a reduction in motivation and arousal and therefore to the "central" component of fatigue. The purpose of the present study was to investigate the effects of a dopamine (DA) noradrenaline (NA) reuptake inhibitor, bupropion (Zyban<sup>TM</sup>), on exercise performance and on the hormonal response to exercise.

**Methods:** Eight healthy well trained male cyclists ( $Watt_{max}$   $397 \pm 15$  W) participated in the study. Subjects completed one maximal exercise test (to determine maximal power output  $Watt_{max}$ ), and two endurance performance tests (time trials) in a double blind randomised cross-over design. Subjects took either placebo capsules (lactose) or  $2 \times 300$  mg bupropion (BUP). Blood samples were collected for adrenocorticotropin (ACTH), prolactin, cortisol, growth hormone, beta-endorphins, and catecholamines.

**Results:** Performance was not influenced by BUP (placebo:  $89 \pm 1$  min; BUP  $2 \times 300$  mg:  $89 \pm 0.7$  min). All hormones increased during exercise in all trials. Cortisol plasma concentrations were significantly higher in the BUP trial at rest, at min 60, and at the end of exercise, while beta-endorphins were higher in the BUP trial at the end of exercise and during recovery, and ACTH at the end of exercise.

**Conclusion:** From the present results, we can conclude that bupropion had a more marked central noradrenergic effect (compared to dopaminergic) on the hormonal response to exercise, but no effect on the outcome of performance.

The past few years there has been growing interest in the role of the central nervous system in the onset of fatigue.

Several hypotheses that neurotransmitters may influence performance have been developed. In particular, the "central fatigue hypothesis" proposed by Newsholme and coworkers<sup>1</sup> revolves around exercise induced increase in serotonin (5-HT) concentrations and the onset of fatigue during prolonged exercise. Serotonin has been linked to fatigue because of its well-known effects on sleep, lethargy and drowsiness, and loss of motivation.<sup>2–3</sup> 5-HT has been shown to increase during acute exercise<sup>4–8</sup> in running rats and to remain high at the point of fatigue.<sup>8</sup>

Dopamine (DA) and noradrenaline (NA) are the other neurotransmitters linked to the "central" component of fatigue because of their well-known role in motivation and motor behaviour.<sup>9</sup> DA has been shown to increase during endurance exercise.<sup>6,8</sup> Animal studies show that at the point of fatigue DA extracellular concentrations are low, possibly due to interaction with brain 5-HT.<sup>8</sup> Many studies in humans have investigated the potential role of 5-HT and/or DA on the outcome of performance, but to date direct proof for the existence of the "central fatigue hypothesis" is scarce. 5-HT reuptake inhibitors (SSRI) have been shown to decrease<sup>10,11</sup> or have no effect<sup>12</sup> on exercise performance. Moreover, no effect on exercise performance in well-trained athletes was observed after supplementation with the noradrenaline reuptake inhibitor (NARI) reboxetine<sup>13</sup> or with a DA precursor.<sup>14</sup> Comparing studies that have used different exercise protocols is not always easy, but most recent studies have used time trial protocols, a very reliable test to measure performance in athletes.<sup>13,14</sup>

Although most recent studies could not detect a difference in exercise performance when selective reuptake inhibitors were used,<sup>12,13</sup> they all showed different hormonal responses depending on the neurotransmitter system triggered by the reuptake inhibitor. The neuroendocrine response to exercise suggested that the drugs had a central effect. NA and DA

have specific effects on the hypothalamic pituitary adrenal axis (HPA): DA in particular has an inhibitory effect on most pituitary hormones except for growth hormone (GH), while the effects exerted by NA depend mostly on the receptor that is activated.<sup>15</sup>

The purpose of the present study was to investigate the effects of the dopaminergic/noradrenergic reuptake inhibitor bupropion (BUP) (Zyban<sup>TM</sup>) on exercise performance and on the neuroendocrine response to exercise, in order to further investigate the role of neurotransmitters on fatigue during exercise.

To our knowledge this is the first study that has evaluated the neuroendocrinological effects of a dopaminergic/noradrenergic reuptake inhibitor during prolonged exercise with this specific protocol. Although BUP is a dual reuptake inhibitor, its potency to inhibit the reuptake of noradrenaline is half that of dopamine according to animal studies.<sup>16</sup> We therefore hypothesise that the central effects of the drug (that is the increase in extracellular neurotransmitters) will be obvious in the peripheral hormonal response to exercise and will be influenced more by the dopaminergic effects of the drug. Moreover, we hypothesise that performance will not be affected by the increase in extracellular neurotransmitters as postulated by the central fatigue hypothesis.

## METHODS

### Subjects

Eight healthy well trained male cyclists (age:  $24.6 \pm 2$  years, height  $183.6 \pm 1.4$  cm, weight:  $76.6 \pm 3.7$  kg,  $Watt_{max}$   $397.25 \pm 15$  W) participated in the study. All subjects read and signed an informed-consent form. This informed-consent

**Abbreviations:** ACTH, adrenocorticotropin;  $\beta$ -E, beta-endorphins; BUP, bupropion; DA, dopamine; GH, growth hormone; NA, noradrenaline; NARI, noradrenaline reuptake inhibitor; PRL, prolactin; RPE, rate of perceived exertion

form and the experimental procedure were approved by the Research Council of the Vrije Universiteit Brussels.

Subjects performed one maximal exercise test to exhaustion (to determine  $\text{VO}_{2\text{max}}$  and maximal power output  $\text{Watt}_{\text{max}}$ ) and two endurance performance tests (time trials). To exclude possible influences of prior exercise, the subjects were requested to avoid any intense or long-lasting physical exercise in the 2 days preceding each experiment. Standard pre-exercise meals were consumed the day before and on the morning of the experiments.

### Maximal exercise test

In all tests the subjects exercised on a cycle ergometer (Excalibur Lode, Groningen, the Netherlands). After resting measurements were collected, the maximal exercise test began with an initial workload of 80 Watts (W) at 70–80 rates per minute (rpm). Thereafter the workload was increased by 40 W every 3 min until the subject was unable to maintain the set power output.  $\text{VO}_2$  was measured throughout the test using an automated system (Metamax, Cortex Biophysik, Germany). During the test lactate and heart rate were measured. Only subjects with  $\text{VO}_{2\text{max}}$  values greater than  $50 \text{ ml kg}^{-1} \text{ min}^{-1}$  were considered for further experiments.

### Time trials

Subjects performed two time trials separated by at least 1 week in order to allow washout of the drug. The two trials were performed in a double blind, randomised, placebo controlled and cross-over design. The night before and on the morning of the time trials (6 h prior the beginning of the test), the subjects ingested two capsules containing either placebo (PLAC) or 300 mg bupropion (BUP). All tests were performed at the same time of day. After a short warm-up (5 min, 100 W) subjects were asked to perform a certain amount of work (equal to about 90 min or 5400 s cycling at 65%  $\text{Watt}_{\text{max}}$ )<sup>12</sup> as fast as possible. This target amount of work was based on the maximal workload ( $\text{Watt}_{\text{max}}$ ) obtained during the graded exercise test to exhaustion and was calculated as follows: target amount of work (J) =  $0.65 \times \text{Watt}_{\text{max}} \times 5400$ .

Subjects started exercise at a preset workload corresponding to 65%  $\text{Watt}_{\text{max}}$  and were thereafter free to increase or decrease the workload as they wished. They were instructed to complete the target amount of work as fast as possible. The measure of performance was the time necessary to complete the target amount of work. During the test the subjects were informed about the percentage of the total preset work that had already been performed. Subjects did not receive any information on workload, pedalling rate, time, or heart rate. During exercise the subjects were allowed to drink water ad libitum. Subjects were also requested to state their rate of perceived exertion (RPE) according to Borg's scale.<sup>17</sup>

### Drugs

The drug and the placebo were prepared in capsules with the same volume, colour, and dimensions. The capsules weighed the same and contained either bupropion (Zyban<sup>TM</sup>, GlaxoWellcome) or placebo (lactose). Capsules were ingested the night before and on the morning of the test (6 h) based on maximal plasma concentrations.

### Blood analysis

Blood samples were collected via an indwelling venous catheter 30 min before the start of the performance test (rest), at 30 min time intervals, at the end of the experiment (when the target amount of work was reached, END), and after 5 min of recovery (REC). Blood samples (20  $\mu\text{l}$ ) only for lactate determination were taken at the hyperaemised

earlobe and assayed by ESAT 6660 lactate (Medingen, Germany). Plasma catecholamines were determined with HPLC EC.<sup>18</sup>

Samples for adrenocorticotropin hormone (ACTH), beta-endorphins ( $\beta$ -E), and catecholamines were collected in pre-frozen 4.5 ml K3 EDTA vacutainer tubes (Beckton Dickinsons Vacutainer System Europe, Plymouth, UK), immediately centrifuged at 3000 rpm (Minifuge 2, Heraeus, Germany) for 10 min, and frozen at  $-20^\circ\text{C}$  until further analysis. Samples for cortisol, growth hormone (GH), and prolactin (PRL) were collected in 8.5 ml vacutainer serum tubes (Beckton Dickinsons Vacutainer System Europe, Plymouth, UK) and kept at room temperature for 1 h before centrifuging at 3000 rpm (Minifuge 2, Heraeus, Germany) for 10 min. Samples were then assayed using radioimmunoassay (RIA) for ACTH and beta-endorphins (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA), PRL (ROCHE Diagnostics, Mannheim, Germany), GH (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden), and cortisol (DiaSorin, Stillwater, USA).

### Statistical analysis

All data are expressed as means  $\pm$  SEM. Statistical evaluation of performance was done using Student's paired *t* test while hormonal and metabolic differences during exercise were analysed using a  $2 \times 5$  way ANOVA and LSD test for post-hoc evaluations. Significance was set at  $p < 0.05$ .

## RESULTS

### Exercise performance

All subjects completed all trials and showed no signs of discomfort due to bupropion. Exercise performance, measured as the time to complete the target amount of work, did not differ between trials (PLAC:  $89 \pm 1$  min; BUP:  $89 \pm 0.7$  min). RPE values from the Borg scale measured during exercise at 15 min time intervals showed no difference between trials. RPE measured at the end of exercise was not different between trials (see table).

### Metabolic data

Lactate concentrations and heart rate (HR), taken at 15 min time intervals, did not differ between trials at all time points (see table).

### Blood hormones

All hormones increased significantly during exercise in both trials. Cortisol plasma concentrations were significantly higher in the BUP trial versus the placebo trial at rest, at min 60, and at the end of exercise. Beta endorphins were significantly higher in the BUP trial compared to the placebo trial at the end of exercise and during recovery. ACTH concentrations were significantly higher in the BUP trial compared to the placebo trial only at the end of exercise. No significant differences were observed in NA, PRL, or GH plasma concentrations between the placebo and the BUP trials (fig 1).

## DISCUSSION

To the best of our knowledge, this is the first paper examining the neuroendocrine effects of a selective DA/NA reuptake inhibitor during prolonged exercise with this particular protocol and subjects. We found that the drug clearly influenced ACTH, cortisol, and beta-endorphin values at rest and during long duration exercise. Performance, however, was not influenced as hypothesised, although most existing literature examining the role of neurotransmitters on exercise performance maintains that catecholamines acting on motor behaviour and motivation<sup>9</sup> increase performance.<sup>8,9</sup>

**Table** HR, lactate, and RPE values in the placebo and bupropion trials

	Rest	15	30	45	60	75	END	REC
<b>HR (bpm)</b>								
Placebo	80±5	154±3	160±3	164±4	169±3	172±5	174±5	133±5
BUP	76±6	158±4	163±3	168±3	172±4	174±4	176±6	131±7
<b>Lactate (mmol l<sup>-1</sup>)</b>								
Placebo	1.2±0.3	1.7±0.4	1.7±0.3	1.9±0.3	2±0.4	2.2±0.3	2.3±0.5	1.9±0.3
BUP	0.8±0.1	1.8±0.4	1.6±0.4	1.6±0.3	1.8±0.4	1.6±0.3	2.2±0.5	1.4±0.2
<b>RPE</b>								
Placebo	0.4±0.4	2.2±0.6	3.2±0.8	4±1	5±0.8	6.4±0.7	8.5±0.5	
BUP	0.3±0.3	1.75±0.6	2.6±0.7	4.8±0.8	5.3±0.7	6.8±0.5	9.5±0.3	

HR, heart rate. No statistical significance was observed at any time point.

Bupropion is a weak but relatively selective inhibitor of DA reuptake. Its potency as an inhibitor of NA reuptake is half that of DA in animals and it shows little affinity for the serotonergic transport system.<sup>16</sup> The major metabolites of BUP, hydroxybupropion (HB) and threohydrobupropion, are weaker inhibitors of DA, 5-HT, and NA reuptake.<sup>16</sup> Electrophysiological studies in animals showed a reduction in the firing rate of noradrenergic neurons in the locus coeruleus in a dose dependent manner,<sup>19</sup> implying that a noradrenergic component may contribute to BUP antidepressant activity. Microdialysis studies, however, show that acute doses of BUP increase extracellular DA in the striatum and nucleus accumbens in a dose dependent manner.<sup>20</sup> It therefore appears that BUP works mainly via the noradrenergic system to produce its antidepressive effects because apparently DA reuptake inhibition occurs at doses that are 7 times higher than the clinical antidepressive dose. This dose is effective in increasing locomotion activity in animals.<sup>16</sup> It is necessary to point out that the pharmacological profile of BUP is different in humans and in animals due to the fact that rodents lack the metabolite HB.<sup>19</sup> In humans the effects of BUP may result from the large concentration of the metabolite which acts as an NA transporter. Due to these findings it is difficult to compare the existing human and animal studies and we should therefore be cautious in the interpretation of our own results.

Subjects ingested BUP 6 h prior to exercise, the time necessary for the metabolite HB to reach maximal plasma concentrations. The differences in the hormonal concentrations observed between the placebo and the BUP trials indicate that the dose administered (2×300 mg) was sufficient to produce an acute central effect. In particular, cortisol, ACTH, and beta-endorphins were higher in the BUP trial compared to placebo. In a previous study we demonstrated that an acute dose of a noradrenergic reuptake inhibitor was able to increase beta-endorphins and ACTH compared to placebo with no effect on cortisol concentrations<sup>13</sup> confirming, at least for ACTH and beta-endorphins, the noradrenergic properties of the drug.

Dopamine has an inhibitory role on most of the pituitary hormones except for GH.<sup>15</sup> Noradrenaline, on the other hand, stimulates the alpha and the beta adrenoceptors in both the central and peripheral nervous systems. Alpha receptors seem to stimulate, while beta adrenoceptors inhibit, GH and ACTH release. Alpha 2 receptor blockade increases PRL release, therefore neuroendocrine regulation by the noradrenergic system depends on the receptors involved.<sup>15</sup>

In the present study resting GH concentrations were more than 3 times higher in the BUP trial (without reaching statistical significance due to the large intra-subject variability), a result comparable to the significantly higher GH resting concentrations after NARI supplementation.<sup>13</sup> Moreover L-DOPA, the precursor of DA, had no effect on GH concentrations compared to placebo in exercising healthy subjects.<sup>14</sup> The GH response to exercise, however, differed

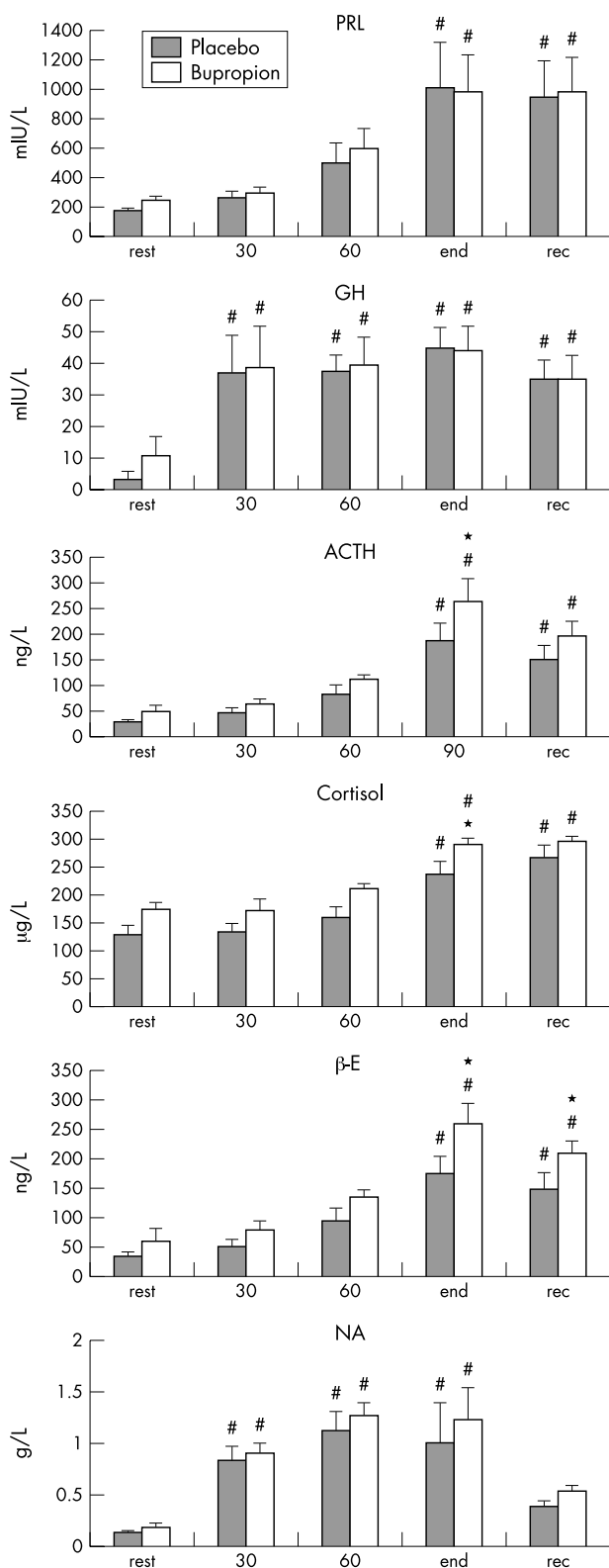
from our previous study where GH decreased during exercise, probably via an autoinhibition mechanism, while in the present study GH continued to increase during exercise. Laakman *et al*<sup>21</sup> were not able to find a significant effect of 200 mg BUP, a dose 3 times lower than the one we utilised, on GH plasma concentrations in healthy volunteers. However, although the difference was not significant, they showed higher GH concentrations after BUP supplementation, due, according to their discussion, to one subject's abnormal response.

In the present study we found no effect of BUP on PRL release. Lower doses (200 mg) were unable to influence PRL secretion in healthy volunteers.<sup>21</sup> We would have expected a decrease in PRL concentrations due to the dopaminergic action of BUP or, if under noradrenergic control, an increase comparable to that found by us after NARI supplementation.<sup>13</sup> The role of PRL as a peripheral marker of dopaminergic and serotonergic activity is questionable. In fact, we previously demonstrated that PRL is decreased, and not increased as expected, by a serotonergic reuptake inhibitor.<sup>12</sup> Moreover, Struder and coworkers found an increase in PRL concentrations after tyrosine supplementation.<sup>10</sup> It could therefore be possible, considering the present and previous results, that PRL is under more complex regulation and could therefore not be considered as a clear peripheral marker of neurotransmitter release.

Lactate and HR were not influenced by the drug in the present study. Apparently the drug has limited peripheral effects and is selective for a central action. Moreover, no subjects reported or presented any side effects of the drug, before, during, or after the experiment. This again could be interpreted as selectivity of action of the drug on central mechanisms.

NA is known to have a primary role in the control of the level of arousal regulation of vigilance consciousness, sleep, anxiety, and reward mechanisms.<sup>22</sup> DA has well-known effects on motor behaviour<sup>19</sup> and therefore is expected to have an enhancing effect on exercise performance. We have already demonstrated that performance is not influenced by a serotonergic reuptake inhibitor in well trained endurance athletes<sup>12</sup> and previous research in humans demonstrated that a dopaminergic precursor, L-DOPA, was unable to improve exercise performance in moderately trained subjects.<sup>14</sup> Tyrosine, the precursor of catecholamines, as well as a NARI were not able to influence exercise performance in well-trained subjects.<sup>10 13</sup>

Several studies have tried to explore the central fatigue hypothesis by manipulating the serotonergic system<sup>4 9</sup> but it seems more than one neurotransmitter system is involved. We therefore studied the possibility of manipulating the noradrenergic/dopaminergic system during a 90 min time trial. Although, according to the "central fatigue hypothesis", a decrease in DA at the end of endurance exercise may be responsible for the "central" component of fatigue,<sup>8 9</sup> in the present study no difference in exercise performance was



**Figure 1** PRL, GH, ACTH, cortisol, beta-endorphins and noradrenaline concentrations in the placebo (closed bars) and bupropion (open bars) trials. \*Statistically different from bupropion; #statistically different from resting values.

observed between placebo and BUP, a noradrenergic dopaminergic reuptake inhibitor. Due to the weak potency in inhibiting the reuptake of DA in humans, the majority of the neuroendocrine effects observed are probably due to the

## Take home message

Bupropion has a marked central noradrenergic effect on the hormonal response to exercise, but no effect on the outcome of performance.

noradrenergic properties of BUP. It would be interesting to study the neuroendocrine response during exercise to a more specific dopaminergic drug.

As for the practical application of this drug, acute administration had no effect on performance, meaning that at the acute dosage used in this experiment, the drug will not influence performance either positively or negatively. This is supported by the new regulation of the International Cycling Federation (UCI) who have removed BUP from the list of forbidden substances (list of 1.1.2003). However, care is still needed since the effects of long-term administration are not known.

In conclusion, although the NA/DA drug had no effect on exercise performance, the hormonal differences observed between the two trials show a marked central noradrenergic effect, most probably due to the presence of the metabolite HB.

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