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# Increases in plasma β-endorphin concentrations during exercise do not contribute to increases in heart rate following autonomic blockade in man

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1 Intrinsic heart rate (IHR: heart rate following autonomic blockade with atropine and propranolol) increases with exercise. The opioid antagonist naloxone has been shown to decrease IHR at rest, raising the possibility that increases in IHR with exercise are β-endorphin related, since β-endorphin concentrations have also been shown to rise during exercise.

2 We examined the effects of naloxone (10 mg) on IHR and plasma β-endorphin levels during aerobic exercise in eight healthy, male subjects in a single blind, crossover study.

3 IHR increased with 25 min bicycling from 97.1 ± 1.4 to 129.7 ± 1.2 beats $min^{-1}$ (mean ± s.e. mean). This rise was not affected by administration of naloxone.

4 Plasma β-endorphin concentration rose from 31.1 ± 3.8 to 94.9 ± 23.9 pg $ml^{-1}$ after 25 min exercise. This exercise-induced rise in β-endorphin concentration was further increased ($P < 0.05$) in the presence of naloxone.

5 Our results confirm a rise in IHR and β-endorphin concentrations with acute exercise but indicate that the changes in IHR are not endorphin-related.

**Keywords** β-endorphin intrinsic heart rate exercise

## Introduction

Intrinsic heart rate (IHR), heart rate following autonomic (β-adrenoceptor and muscarinic) blockade, increases with exercise (Jose *et al.*, 1970). The cause of this increase is unknown. Possible mechanisms include exercise-induced rises in central body temperature (Pickering, 1958) and/or neural-humoral influences other than those mediated by the autonomic nervous system (Jose *et al.*, 1970). These neural-humoral influences include exercise-induced rises in angiotensin II, aldosterone, cortisol and β-endorphin, all of which are further enhanced in the presence of naloxone (Staessen *et al.*, 1988).

It is well established that circulatory levels of opioids increase with exercise (Brooks *et al.*, 1988; McMurray *et al.*, 1987; Rahkila *et al.*, 1988). Opioid antagonism with naloxone further increases these levels (Bramnert, 1988; Staessen *et al.*, 1988) but has not been found to alter heart rate in the presence of intact neural innervation. To date no studies have examined the effect of naloxone on IHR changes during exercise. Studies in our laboratory have demonstrated that naloxone reduced resting IHR in untrained subjects. A period of constant exercise training also reduced resting IHR but in the trained state naloxone produced no additional effect (Kingwell *et al.*, 1989). These results would be consistent with a stimulatory affect of β-endorphins on IHR and a training effect reducing β-endorphins (and hence IHR) levels. The possibility that exercise-induced IHR changes are due to rises in circulatory β-endorphin is examined in the present study.

## Methods

### *Subjects*

Eight healthy male participants, aged between 18–23 years, were recruited for the present study. All were non-smokers and none were heavy drinkers. Each subject's maximum work capacity ($W_{max}$) and maximum

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oxygen consumption ($VO_{2\ max}$) was determined using a graded sprint test on a cycle ergometer (Ergoline 900) which involved starting at 0 watts and increasing by 20 watts each minute until further increase in workload (watts) was prevented by fatigue (Jennings *et al.*, 1979). Gas analysis was performed using a Medical Graphics Corporation 2001 CAD/Net Cardiopulmonary Exercise System.

*Study protocol*

Studies were conducted on two occasions separated by a minimum of 2 days. Subjects arrived at the laboratory at 13.30 h on the day of each study having fasted for at least 4 h. Heart rate was recorded from lead II of the ECG on a Spacelab Patient Monitor (series 90603). Blood pressure was recorded at the brachial artery every minute throughout each study by one of two trained nurses using a sphygmomanometer. The antecubital vein was cannulated for i.v. infusion of drugs and blood sampling. Propranolol (0.2 mg $kg^{-1}$) was infused over 10 min followed by atropine (2 mg total) over a further 10 min. A Valsalva manoeuvre was then performed to demonstrate the effectiveness of propranolol and atropine in producing cardiac autonomic blockade (Jennings *et al.*, 1979). Four subjects were randomly allocated to receive naloxone (10 mg over 10 min) during the first session while the other four received naloxone during the second session. Subjects had no knowledge as to whether naloxone was administered during the first or second session. Following the drug infusions subjects were instructed to cycle on a stationary ergometer (Ergoline 900) for 25 min at 50% of their predetermined $W_{max}$ after a 5 min warm-up period at 40 watts. Each exercise session was followed by a 5 min cool down period. A further Valsalva manoeuvre was performed at the end of the exercise session to determine the continuing presence of cardiac autonomic blockade.

Blood samples (10 ml) were collected into 10,000 kiu of aprotinin in heparin coated tubes. Samples were taken both before and after the infusion of the drugs and at 5, 15 and 25 min during exercise and at 5 min post-exercise. Blood was centrifuged at 2500 rev $min^{-1}$ for 10 min and the plasma stored at −20° C until extraction and assay for β-endorphins.

*Extraction and radioimmunoassay of plasma β-endorphin concentrations*

Samples were assayed at Prince Henry's Institute of Medical Research, Melbourne. β-endorphin was extracted from plasma onto Vycor glass (Lees *et al.*, 1971). Briefly, 1.5 ml aliquots of plasma were shaken with 60 mg Vycor for 30 min on an IKA Vibrax agitator. Samples were centrifuged (2500 rev $min^{-1}$, 5 min), plasma aspirated and the Vycor washed with 1.0 ml distilled water. Centrifugation (2500 rev $min^{-1}$, 5 min) of the suspended Vycor was followed by aspiration of the water wash. Adsorbed peptide was eluted from the Vycor glass by 30 min shaking with 2 ml 80% methanol in 0.1 ml HCl. The elute was dried in a Savant Rotary vacuum apparatus and samples reconstituted to 750 μl with r.i.a. buffer.

β-endorphin was measured by a modification of the method previously described (Engler *et al.*, 1988). Synthetic human β-endorphin was used as standard and the assay performed under disequilibrium conditions to maximize assay sensitivity. Detection limit of the assay was 2 pg per tube. Plasma extracts of 200 μl volume (equivalent to 400 μl plasma) were assayed in duplicate, so that the detection limit for plasma was 5 pg $ml^{-1}$.

*Drugs*

Atropine sulphate (Astra), naloxone hydrochloride (Boots Co.), propranolol hydrochloride (ICI).

*Statistical analysis*

Statistical analysis of the heart rate data was by comparison of regression lines and that of β-endorphin levels by three-way ANOVA and the Student Newman-Keul's test. $P < 0.05$ was adopted as the criterion of statistical significance.

*Ethical approval*

Prior ethical approval for this study was obtained from the Alfred Hospital Ethics Committee.

## Results

The Valsalva manouevre performed directly after the infusion of propranolol and atropine confirmed the presence of cardiac autonomic blockade by the absence of heart rate changes including the lack of tachycardia during the steady state phase of the response and of bradycardia during the overshoot phase. Results from the manouevre performed at the end of each exercise session confirmed the continuing presence of autonomic blockade during each session in all cases.

*Heart rate changes*

The mean resting heart rate of the eight subjects increased from 70.0 ± 2.5 (mean ± s.e. mean) to 90.0 ± 3.1 beats $min^{-1}$ in the presence of atropine and propranolol. A further increase in IHR, by approximately 6 beats $min^{-1}$, was observed during the 5 min warm-up session which was further elevated (from 97.1 ± 1.4 to 109.4 ± 1.2 beats $min^{-1}$) 1 min into exercise at 50% $W_{max}$. A time-related gradual increase in IHR was then observed with continued exercise (Figure 1). Naloxone had no effect on this pattern of IHR changes with exercise (slopes: 0.71 *vs* 0.79, $P > 0.05$, Figure 1).

*Circulating plasma β-endorphin concentrations*

Propranolol and atropine, given in concentrations sufficient to induce cardiac autonomic blockade, had no effect on plasma β-endorphin concentrations ($P > 0.05$). A further infusion of naloxone had no influence on resting plasma β-endorphin concentration. These levels, however, were observed to rise with

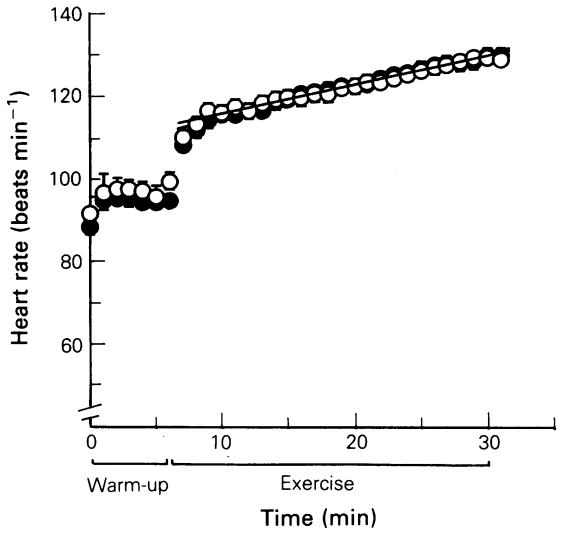


**Figure 1** Mean intrinsic heart rate values during the warm-up period and exercise in the absence (○) and in the presence (●) of naloxone. Results are given as mean ± s.e. mean of eight observations. The slopes of the regression lines (0.71 *vs* 0.79 respectively) during the exercise phase were not significantly different.

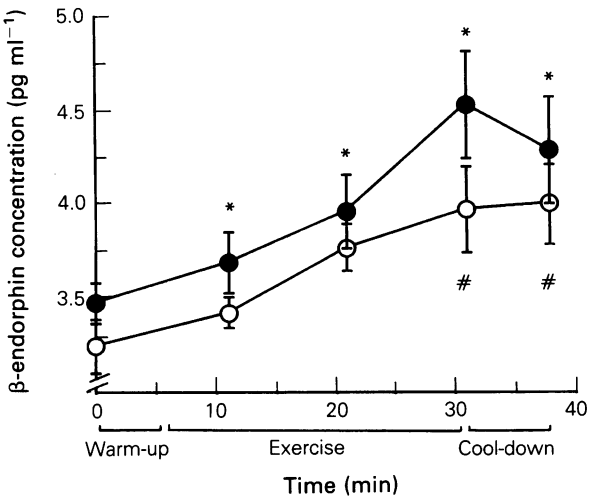


**Figure 2** Mean plasma β-endorphin concentrations at rest, during exercise and 5 min post-exercise in subjects after autonomic blockade in the absence of (○) and after administration of (●) naloxone. * denotes a statistically significant difference (Student's Newman-Keul's test; $P < 0.05$) between this and all other values within the same group. # denotes a statistically significant difference (3-way ANOVA; $P < 0.05$) between the two groups.

exercise. Plasma β-endorphin concentrations obtained at 15 and 25 min ($P < 0.05$) during exercise and 5 min post exercise were significantly elevated when compared with resting (pre-exercise) values and to levels 5 min into exercise (Figure 2). Naloxone further promoted this increase 5, 15 and 25 min into exercise ($P < 0.05$; Figure 2).

## Discussion

Parasympathetic and sympathetic blockade in this study was achieved using atropine and propranolol to block muscarinic receptors and β-adrenoceptors respectively. This resulted in an increase in heart rate at rest due to interruption of tonic vagal control. Other neural influences, for example peptidergic or purinergic inputs were not blocked. α-adrenoceptors were also not blocked.

In the present study, in keeping with results from previous studies (Jose *et al.*, 1970), heart rate increased with exercise following autonomic blockade. This increase cannot be explained by incomplete autonomic blockade as Valsalva manoeuvres performed after each exercise session confirmed the effectiveness of the parasympathetic and sympathetic blockade. An abrupt increase in IHR occurred immediately on commencement of exercise followed by further gradual elevation throughout the duration of exercise. Naloxone, 10 mg infused over 10 min, had no effect on either the abrupt or gradual exercise-induced rise in IHR.

The rise in plasma β-endorphin concentration has been suggested to be related to exercise intensity so that light to moderate aerobic exercise does not exert any influence on circulating β-endorphin whereas exercise intensities greater than 75–80% of an individual's $VO_{2\ max}$ quickly elevates these levels (McMurray *et al.*, 1987; Rahkila *et al.*, 1988). The effect of autonomic blockade on exercise-induced increases in plasma concentrations of β-endorphins has, however, not previously been studied. Our data shows that a workload of 50% $W_{max}$ (calculated as an exercise intensity of 50% $VO_{2\ max}$ pre-autonomic blockade) was sufficient to raise plasma β-endorphin concentrations. The elevation of β-endorphin concentrations appears to be a gradual one, increasing through the warm up period into the exercise phase.

Naloxone has been found to stimulate α-melanocyte-stimulating hormone (MSH) and β-endorphin release from rat pituitaries (Al Zein *et al.*, 1986), and adrenal corticotrophin hormone (ACTH) and β-endorphin release during stress in humans (McCubbin *et al.*, 1989). Similarly, β-endorphin concentrations were found to be significantly elevated following naloxone administration during exercise in the present study; this elevation was detected throughout the course of the experiment. The increase in β-endorphin concentrations following naloxone administration confirms that a sufficient dose of the antagonist was given to effectively antagonise opioid receptors *in vivo*.

Naloxone is primarily a μ-receptor antagonist, with relative affinities of 0.85, 0.06 and 0.09 for the μ, γ and κ receptors respectively while β-endorphin has a high affinity for both μ (1.0) and γ (0.9) receptors (Paterson *et al.*, 1983). There is little documentation on the characterisation of opioid receptors in cardiac tissue. β-endorphin has been demonstrated to reduce left ventricular systolic and diastolic pressure in the rat isolated heart preparation (Lee *et al.*, 1984) probably by its action on pre-synaptic inhibitory μ-receptors located on excitatory non-adrenergic, non-cholinergic (NANC) nerves present in the mammalian heart (Mantelli *et al.*, 1990; Rubino *et al.*, 1990). Although the NANC were not inhibited in our study, since no effect in heart rate was seen with the administration of naloxone, it would appear that an insufficient concentration of this opioid antagonist was given to induce a local effect on the heart.

In 1988, Staessen *et al.* reported that exercise-induced increases in plasma angiotensin II, aldosterone, cortisol and the catecholamines, noradrenaline and adrenaline, were higher in the presence of naloxone than saline in the neurally intact human. Whether the plasma levels of any of these hormones were similarly elevated in our model was not examined. However, since opioid antagonism did not alter exercise-induced rises in IHR, we can conclude that IHR is not endorphin-related nor a result of possible further increases in the other hormones.

## Conclusion

From our data it is clear that β-endorphin concentrations increase with exercise in the presence of autonomic blockade. Similarly, intrinsic heart rate increases with acute aerobic exercise. However, while β-endorphin levels were further elevated in the presence of naloxone, this opioid antagonist had no effect on IHR changes. We thus conclude that the rise in IHR during exercise is not influenced by circulating endorphins.

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