The effects of a peripherally selective α_2 -adrenoceptor antagonist, MK-467, on the metabolic and cardiovascular response to exercise in healthy man

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- 1 A double-blind, placebo controlled study has been conducted to investigate the consequences of i.v. treatment with MK-467, a peripherally selective α_2 -adrenoceptor antagonist in exercising healthy male subjects. In particular, the effects on blood pressure, heart rate, circulating catecholamines (noradrenaline (NA) and adrenaline (A)), insulin, glucose and free fatty acids (FFA) were determined.
- 2 Exercise produced increases in catecholamines, blood pressure and heart rate. FFA increased at the start of the exercise but then declined as exercise progressed.
- 3 MK-467 significantly increased NA, in a dose-dependent manner, before and during exercise without altering A. Blood pressure and heart rate were not affected by drug treatment.
- 4 The insulin and FFA response to exercise was significantly enhanced by MK-467 although glucose was unaltered by drug.
- 5 It is concluded that both pre- and post-junctional peripheral α_2 -receptors play an important role in the metabolic response to exercise in man.

Keywords noradrenaline lipolysis insulin vital signs

Introduction

Following the discovery in the early 1970s of prejunctional α -adrenoceptors [1], it was thought that physiological relevance was limited to modulation of the release of noradrenaline (NA). The term 'autoreceptor' was coined to describe functionally this novel class of receptor which subsequently was subclassified as an α_2 -receptor [2]. Since then, the use of highly selective agonists and antagonists has demonstrated the presence of such receptors at post-junctional sites including adipose tissue [3], platelets [4], and pancreas [5]. These findings have precipitated the need for a pharmacological rather than anatomical classification.

Evidence that selective antagonism of the α_2 -receptor may augment insulin secretion has generated much research interest in the potential role of this class of drug in diabetic therapy. Robertson *et al.* [6] showed that phentolamine-induced α -adrenoceptor blockade increased the insulin response to glucose in both normal and diabetic subjects. It was thought that the tonic increase in insulin secretion was primarily a result of reduced α -adrenergic stimulation since simultaneous α -adrenoceptor blockade did not influence the response.

More recent studies, however, with selective and potent α_2 -adrenoceptor antagonists (e.g. idazoxan) in both normal and diabetic subjects have failed to corroborate the phentolamine data [7].

In adipocytes, α_2 -receptor agonists inhibit lipolysis and hence release of free fatty acids (FFA), whereas stimulation of adenylate cyclase via β -receptors promotes lipolysis. An expected net consequence of α_2 -adrenoceptor blockade would be increased circulating FFA and insulin together with an increased heart rate (see Figure 1). It might be expected that increased sympathetic drive such as under exercise conditions would exaggerate some or all of these anticipated effects, providing a model for investigating the effects of α_2 -adrenoceptor blockade. Investigation of these effects, however, with available α_2 -adrenoceptor antagonists, such as yohimbine [8] and idazoxan [7], is made complex by the potential of these agents to block central α_2 -receptors, altering both sympathetic outflow and other neuroendocrine and behavioural functions [9].

The recent availability of MK-467 (previously known

Figure 1 An overview of the adrenoceptor with specific reference to insulin secretion, lipolysis and cardiovascular physiology. Also illustrated are the addressed hypotheses concerning anticipated consequences of peripheral α_2 -adrenoceptor blockade.

as L-659,066), a spirocyclic-substituted benzofuroquinolizine and a potent and selective α_2 -receptor antagonist which does not penetrate the blood brain barrier [10], has made the investigation of this hypothesis easier. Pre-clinical studies have shown MK-467 to have a low brain/plasma ratio (0.038) conferring the novel attribute of peripheral selectivity which functional studies have confirmed in both animal [10] and man [11]. Furthermore, although structurally related to yohimbine, MK-467 has been shown to have up to 5 times greater selectivity for α_2 - over α_1 -receptors in pre-clinical models [10].

The primary hypotheses behind the present study are summarised in Figure 1 and the resultant objectives were to evaluate the effect of MK-467 on the cardiovascular and metabolic responses to exercise in normal man. The potential therapeutic implications of selective peripheral α_2 -adrenoceptor blockade in enhancing the metabolic response (e.g. increased lipolysis, insulin release) could then be evaluated against the anticipated cost of increased cardiovascular stimulation, without the complicating influence of central α_2 -adrenoceptor blockade on sympathetic outflow.

Methods

Nine healthy male volunteers (19–28 years, mean 21.8) were enrolled into the study after ethics committee approval and each subject's written informed consent. On 3 separate study days, subjects received either placebo or active drug at one of two doses (low, L, and high, H). The infusion regimens were chosen on the basis of earlier safety and pharmacokinetic studies in man and receptor binding data and were calculated to achieve plasma levels of 4x and 13x *in vitro* K_d , assuming 90% protein binding, i.e. 90 ng ml⁻¹ (L) and 440 ng ml⁻¹ (H). In order to achieve the required levels during exercise, treatment was given, via a venous cannula, as a bolus over 15 min (1.5 mg-L, 7.5 mg-H) followed by a maintenance infusion throughout the period of exercise (10 µg min⁻¹-L, 50 µg min⁻¹-H). Plasma drug assay confirmed the success of this design (results shown in Table 1). Treatment order was under double-blind conditions and followed a semi-randomised schedule arranged so that each subject received drug in ascending dosage. All subjects had fasted and were confined to bed overnight in the

Table 1 Mean plasma concentrations of MK-467 vs expected levels, calculated according to the infusion design, at the end of bolus infusion and at the start of the standardised exercise (ng ml^{-1} , $\pm$ s.d.; $n = 8$)

Dose group	End of bolus		Start of exercise	
	Expected	Actual	Expected	Actual
Low	84	165 $\pm$ 25	90	114 $\pm$ 25
High	532	596 $\pm$ 79	440	418 $\pm$ 63

research unit for the 10 h prior to each infusion. Experiments were always conducted at the same time of day, with a washout period of at least 14 days.

On each study day, bilateral cannulae were inserted into superficial brachial veins; one was used for drug infusion, the other for blood sampling. The subjects then rested supine for an additional hour prior to start of treatment. After the bolus dose and approximately 15 min of maintenance infusion, the subject was seated on an electrically braked cycle ergometer and, following an acclimatisation period of approximately 5 min, began pedalling at 55–60 rev min^{-1} . For the first minute of exercise there was no externally applied load, thereafter the load was increased to 20 watts and incremented by 20 watts each minute. The exercise was terminated when the respiratory exchange ratio reached a value of unity (approximately 70% of maximal oxygen uptake, [12]). On completion of the exercise test, the subjects returned to the supine position and heart rate and blood pressure were monitored until recovery to pre-exercise resting levels. At this point the infusion ceased and the cannulae were withdrawn. The total duration of drug infusion averaged 90 min after completion of the 15 min bolus.

The respiratory exchange ratio (R , $= \dot{V}\text{CO}_2/\dot{V}\text{O}_2$) was calculated from measurements of oxygen uptake ($\dot{V}\text{O}_2$) and carbon dioxide output ($\dot{V}\text{CO}_2$) using computerised indirect calorimetry (P.K. Morgan Inc.). The electrocardiogram was recorded continuously throughout the whole of the study period from bipolar chest leads [13]. Blood pressure was estimated non-invasively (Infrasonde) each minute at the completion of each workload, including the first minute at zero load, and at 2 min

intervals during the pre-exercise and recovery periods.

Blood samples were taken at rest and at 2 min intervals with prompt centrifugation and subsequent assay for catecholamines, measured by h.p.l.c. with electrochemical detection [14]. Free fatty acids (enzymatic assay with Cobas Bio analyser), 3-hydroxybutyrate, glycerol (perchloric acid extraction followed by Cobas Bio analyser with fluorimetric attachment), glucose (Yellow Springs Glucose analyser), and insulin (double antibody radioimmunoassay) were also measured. Results are expressed as significant if statistical analysis rendered a P value of 0.05 or less. Analysis of drug and time effects was conducted using analysis of variance (BMDP statistical software program 2V) with appropriate paired t -testing for individual timepoints. For clarity, only data up to 10 min of exercise have been used in the analysis, although some volunteers continued for several further minutes before reaching the exercise endpoint of R equal to unity.

Results

During the third test day, one volunteer experienced reversible tachycardia and hypertension (maximal blood pressure 140/100 mm Hg, and heart rate 110 beats min^{-1}) prior to start of exercise which necessitated discontinuation of the infusion. He was subsequently found to be on high dose drug. Another volunteer experienced tachycardia on placebo (first test day) and was withdrawn from the study. Data from these subjects were not included in the analysis. Only results for completed exercise periods are included in the analysis.

Table 2 shows the heart rate and blood pressure results during exercise on each of the study days. No significant drug effects on either of these parameters, at rest or during exercise were found.

Plasma NA, insulin and FFA concentrations throughout each study period, both before and during exercise, are shown in Figure 2. Generally, as expected, NA levels rose steadily as exercise progressed (Figure 2a). Both low and high dose of drug caused a significant increase in NA at 30 min while subjects were

Table 2 Mean blood pressure and heart rate (range, $n = 8$) immediately pre-infusion commencement, 30 min after start of infusion (supine), at the start of exercise (on the ergometer, no pedalling) and after 10 mins exercise

	Baseline	30 min	Pre-exercise	10 min exercise
<i>HR (beats min^{-1})</i>				
PL	59 (49–76)	60 (49–73)	73 (61–98)	139 (103–153)
LO	59 (40–75)	62 (42–92)	82 (62–111)	146 (136–162)
HI	58 (38–70)	60 (39–72)	76 (57–83)	150 (124–167)
<i>BP (mm Hg)</i>				
<i>Diastolic</i>				
PL	71 (53–83)	70 (58–84)	69 (57–89)	77 (63–104)
LO	69 (59–82)	68 (53–80)	76 (57–97)	74 (64–93)
HI	69 (59–79)	71 (57–83)	73 (69–88)	80 (61–97)
<i>Systolic</i>				
PL	121 (107–131)	122 (111–140)	118 (97–137)	165 (109–201)
LO	121 (102–138)	124 (106–144)	120 (104–137)	161 (122–185)
HI	119 (100–128)	124 (103–136)	122 (98–141)	181 (145–201)

PL, placebo; LO, low dose; HI, high dose.

Figure 2 a) Mean plasma noradrenaline concentration after MK-467 infusion at low (LO, $\blacktriangle$) or high (HI, $\blacktriangledown$) dose, or placebo (PL, $\blacksquare$, $n = 8$, $\pm$ s.e. mean). * < 0.05 , ** < 0.01 , P values indicating significant difference from placebo for individual time points prior to and during exercise.

b) Mean plasma insulin concentrations after placebo (PL), and MK-467 infusion at low (LO) or high (HI) dose ($n = 8$, $\pm$ s.e. mean). * < 0.05 , P values indicating significant difference from placebo.

c) Mean plasma free fatty acid levels (expressed as % baseline) after placebo (PL), and MK-467 infusion at low (LO) or high (HI) dose ($n = 8$, $\pm$ s.e. mean). * < 0.05 , P values indicating significant difference from placebo. 'PE' indicates post exercise recovery period.

still supine. Following repositioning on the exercise bike (35 min) and the start of pedalling against no resistance, the expected 'postural' increase NA was noted on placebo, with a dose-related enhancement of this effect by MK-467 ($P < 0.05$). During incremental exercise, the difference between MK-467 and placebo was maintained. The A response to exercise was not affected by MK-467 (see Table 3).

During placebo, an abrupt increase in plasma insulin concentration (Figure 2b) was observed around the start of exercise which then declined back to baseline levels as exercise load increased. This acute rise in

Table 3 Mean plasma adrenaline changes from baseline (BL, immediately pre-infusion) at 30 min post infusion start (supine), upright on bike (35 min) and at 2 min intervals through exercise. The data in the BL column are means of absolute data, other data are mean of individual change from baseline ($n = 8$, nmol l^{-1})

	BL	Exercise period						
	30 min	35 min	2 min	4 min	6 min	8 min	10 min	
PL	0.35	-0.05	0.06	0.18	0.27	0.23	0.30	0.57
LO	0.35	-0.01	0.05	0.25	0.28	0.36	0.40	0.62
HI	0.36	0.02	0.04	0.31	0.23	0.25	0.26	0.47

insulin was significantly enhanced by MK-467, again in a dose-related manner ($P < 0.01$). The overall profile of the response remained unaltered by α_2 -adrenoceptor blockade, although, during high dose MK-467, insulin concentrations were greater than baseline throughout most of the exercise period, whereas, during placebo, baseline concentrations were reached by 6 min of exercise. In spite of the enhanced insulin response, plasma glucose remained relatively stable during exercise and was unaffected by treatment with MK-467 (Table 4).

Due to the large inter-subject variation in plasma FFA levels, data were expressed as % of baseline (Figure 2c). During the placebo exercise period, an increase in plasma FFA was observed at the start of exercise, resembling the insulin data. The effect of MK-467 was to increase significantly plasma FFA while supine, followed by further increases during exercise with the changes being dose-related ($P < 0.05$). It is interesting to note that MK-467 was associated with a rise in plasma FFA levels prior to the start of exercise but this change did not reach statistical significance.

The data from assay of plasma β -hydroxybutyrate and glycerol, whilst showing a similar pattern to the FFA, did not show a clear dose-response relationship (Table 5). MK-467 did, however, produce a statistically significant enhancement of the exercise-induced increase in each of the lipid metabolites ($P < 0.05$, low dose).

Discussion

It has been shown that α_2 -receptor antagonism with yohimbine in man results in elevation of plasma NA without significant effect of A [8]. This effect of yohimbine was also associated with increases in blood pressure, enhanced sympathetic reflexes and effects on mood. These results suggested blockade of central α_2 -receptors, although peripheral effects were also plausible.

Under exercise conditions, Struthers *et al.* [15] showed, using idazoxan, that exercise-associated increases in heart rate, and catecholamine and growth hormone concentrations were enhanced by α_2 -adrenoceptor antagonism. Growth hormone at rest however

was unaffected by idazoxan. These important studies of the physiological role of α_2 -receptors were confounded by the potential site of action of the antagonists used, allowing only speculative discrimination of central vs peripheral α_2 -mediated effects. Nevertheless animal studies have shown that the NA effect of idazoxan is far stronger when the drug is infused intravenously than when injected into cerebral ventricles [16]. Thus, it was thought more likely that the catecholamine responses to α_2 -adrenoceptor antagonism are due to removal of a tonic control of peripheral NE release, both at rest and during exercise [15, 17]. Confirmation of this hypothesis requires a model for selectively stimulating or blocking peripheral α_2 -adrenoceptors.

It has been demonstrated in pre-clinical studies that MK-467 is unable to cross the blood brain barrier to any significant extent [10]. This has been confirmed functionally in man in a study of the effects of MK-467 on the responses to intravenous clonidine [11]. MK-467 was shown to inhibit the peripheral effects of clonidine (reduced plasma NA and insulin, increased plasma glucose) while the central effects (sedation, reduced salivary flow, increased plasma growth hormone) remained unaltered. In the present study, MK-467 administration resulted in a dose-related increase in NA compared with placebo at rest which persisted throughout a standardised exercise period. These data confirm that α_2 -adrenoceptor antagonist-associated increases in NA are peripherally mediated and are probably accounted for by the removal of local tonic control of NA release. No drug-associated effects on heart rate were found despite the presence of enhanced plasma NA concentrations. Heart rate and blood pressure were least likely to be affected by drug according to the study hypothesis (Figure 1). Idazoxan, however, has been shown to cause augmentation of exercise-induced increases in heart rate, NA and A [15]. The peak mean NA concentrations were augmented by >2.5 fold after idazoxan against an increase of <1.5 after MK-467. This discrepancy is most likely due to idazoxan acting centrally as well as in the periphery and hence influencing plasma NA at more levels than MK-467; moreover it may explain the absence of effect of MK-467 on heart rate. The lack of effect MK-467 on A in the present study strongly suggests that α_2 -modulation of A release is central rather than peripheral, and, therefore, that the release of A from the human adrenal gland during rest and dynamic exercise is not under control of peripheral α_2 -receptors.

Table 4 Mean plasma glucose concentrations following infusion of placebo (PL) low dose (LO) and high dose (HI) MK-467 while subjects were supine (0, 30 min), on moving to the bike (35 min) and during first 10 min of exercise ($n = 8$, mmol l⁻¹)

Time (min)	PL	LO	HI
0	4.65	4.71	4.87
30	4.67	4.58	4.37
35	4.58	4.75	4.38
2	4.66	4.87	4.62
4	4.50	4.80	4.44
6	4.66	4.82	4.39
8	4.41	4.91	4.29
10	4.20	4.88	4.55

Table 5 Effects of exercise on plasma β -hydroxybutyrate and glycerol levels during exercise. Assay results are expressed as % baseline to correct for inter-individual differences. Data shows mean % change from baseline (0 h—immediately pre-bolus dose, mmol l⁻¹) at immediately pre-exercise start (Pre-ex) and peak increase during exercise (Peak-ex)

Time	β -hydroxybutyrate			Glycerol		
	PL	LO	HI	PL	LO	HI
Baseline	71.3	86.8	113.0	70.8	64.4	55.6
Pre-ex	84.8%	141.0%	113.2%	73.1%	68.9%	57.6%
Peak-ex	98.4%	159.7%	140.3%	119.0%	146.5%	131%

Interest in the metabolic effects of the adrenergic system was stimulated by observations that phentolamine administration was associated with enhancement of insulin secretion in both normal and diabetic subjects [6, 18], suggesting an alternative mechanism for reducing blood glucose concentration in diabetics.

It might be expected that removal of tonic inhibition exerted by α_2 -receptors in pancreas and adipose tissue would lead to increased circulating levels of insulin and FFA, which are an important substrate for skeletal muscle under exercise conditions [19]. In the present study, under conditions of increased endogenous sympathetic drive (i.e. exercise), MK-467 administration resulted in a dose-related enhancement of the insulin and lipolytic response to exercise. The evidence for enhanced lipolysis is supported by augmentation of the exercise-associated increase in plasma glycerol. Enhancement of FFA turnover by MK-467 is also suggested by the effect on β -hydroxybutyrate.

Irrespective of treatment, mean plasma insulin rose during the first 2 min on the exercise cycle, and declined subsequently as exercise load increased. This early increase may well have been due to a postural-associated change in sympathetic tone as the subjects moved from a supine to a seated position on the cycle.

The general decline in insulin during the latter stages of exercise is consistent with published data [20].

The lack of any drug associated effect on glucose is of particular interest in the light of insulin responses. It is probable that the many levels of compensation in the control of blood glucose counterbalanced the effects of α_2 -adrenoceptor antagonism in this study.

Conclusion

MK-467 significantly increased NA before and during exercise without altering A.

The insulin and lipolytic responses during exercise were enhanced in the presence of MK-467. It is possible that these effects were a result of increased NA levels unopposed by α_2 -adrenoceptor mediated inhibition.

To conclude, it appears that both pre- and post-junctional peripheral α_2 -adrenoceptors play an important role in the metabolic response to exercise in healthy man.

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