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Effects of physiological and pharmacological variation of sympathetic nervous system activity on plasma non-esterified fatty acid concentrations in man

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1 The consequence of the sympatholytic effect of clonidine (α_2-adrenoceptor agonist) was compared with the effect of a physiological inhibition of sympathetic nervous system activity (change from upright to supine position) on plasma catecholamine and non-esterified fatty acid (NEFA) concentrations in overnight fasting healthy men.

2 Clonidine (150 μg orally) administered in upright position induced a significant reduction of plasma noradrenaline and NEFA concentrations. A change from upright to supine position which provoked a more marked decrease in plasma noradrenaline concentrations induced a weak increase in plasma NEFA concentrations.

3 The modification of plasma NEFA and catecholamine concentrations brought about by standing up was studied after placebo or yohimbine (α_2-adrenoceptor antagonist) administration. With placebo, standing up promotes a 100% increase in plasma noradrenaline concentrations (measured 5 and 15 min after rising) and a weak transient decrease in plasma NEFA concentrations (5 min after rising). In the supine position, yohimbine increased plasma noradrenaline and NEFA concentrations by about 100% and 55% respectively. Standing after yohimbine administration promoted large increases in plasma noradrenaline and NEFA concentrations.

4 These results indicate that a reduction of sympathetic nervous activity is not associated with a decrease of plasma NEFA concentrations and argue for a role of α_2-adrenoceptors in the NEFA mobilization from adipose tissue after sympathetic nervous system activation in man.

Keywords catecholamines non-esterified fatty acids clonidine yohimbine sympathetic nervous system activity man

Introduction

The sympathetic nervous system and catecholamines are of importance for the control of lipolysis in adipose tissue, particularly in humans where the adipocytes are weakly sensitive to the lipolytic effect of other agents. Moreover, human fat cells possess both α_2- and β-adrenoceptors mediating antagonistic effects on adenylate cyclase activity, cAMP production and lipolysis [1–4]. The balance between these adrenergic receptors appears to be essential for the *in vitro* control of the final lipolytic response initiated by catecholamines in human adipocytes [5–7].

The physiological relevance of the *in vitro* studies performed on isolated adipocytes is not clearly understood. Attempts to evaluate the physiological role of the sympathetic nervous system suggested that, in the rat, only noradrenaline is able to stimulate the lipolytic process in the adipose tissue *in vivo* [8, 9]. Investigations made to demonstrate the role of the sympathetic nervous system and of circulating catecholamines in the mobilization of lipids in man have led to controversial data. Short-term fasting (12 h) induces a classical increase in NEFA but does not modify plasma

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catecholamine levels [10]. Longer fasting periods (84 h) also failed to increase plasma noradrenaline whereas plasma NEFA were greatly increased [10]. On the other hand, Keller *et al.* [11] showed that the infusion of noradrenaline resulted in an increase in plasma NEFA in overnight-fasted humans. This effect was abolished by β-adrenoceptor blockade (propranolol) and transiently enhanced by α-adrenoceptor blockade (phentolamine). Liggett *et al.* [12] also found that plasma NEFA concentrations could be enhanced by adrenaline infusion in man. Pharmacological trials to enhance noradrenaline concentrations were conducted by us [13, 14] and others [15] using the α_2-adrenoceptor antagonist, yohimbine. Its administration promoted lipomobilization in fasting subjects by activation of the sympathetic nervous system (indicated by an increase in plasma noradrenaline concentrations) and stimulation of fat cell β-adrenoceptors [13, 14].

The present study was performed in humans (1) to determine whether physiological or pharmacological reduction in sympathetic nervous system activity and noradrenaline secretion (through activation of the baroreflex or stimulation of α_2-adrenoceptors respectively) influence plasma NEFA concentrations and 2) to study the effect of α_2-adrenoceptor blockade on sympathetic nervous system activity and plasma NEFA concentrations during baroreflex inactivation.

Methods

Clinical protocols

Informed consent was obtained from seven normal healthy male volunteers (mean age: 31 ± 3 years, mean body mass index (BMI): 22.8 ± 0.3) and the experimental protocol was approved by the Regional Ethical Committee. All subjects had a normal medical status, with normal glucose tolerance and were taking no medications. They were on their normal diet (7500–9200 kJ day^{-1}) before the experiments and presented a stable normal weight for at least 1 month before the beginning of the study. All experiments began at 08.30 h after an overnight fast. An indwelling teflon catheter was placed in the antecubital vein. Diastolic and systolic blood pressure and heart rate were measured using an automatic device (Dinamap).

A protocol was conducted in order to compare the effects of a physiological or pharmacological reduction of sympathetic nervous system activity on plasma NEFA and catecholamine concentrations. In a first trial, the subjects were kept in an upright position for 30 min and, after a blood sample had been taken, they were placed in the supine position for 1 h after which another blood sample was taken. This manoeuvre induces baroreceptor activation and leads to a reduction in sympathetic nervous system activity. Heart rate and blood pressure were determined just before each blood collection. Seven days later, the effect of clonidine administration was studied. For that, the subjects were kept in an upright position for 30 min. A blood sample was taken before they received orally 150 μg clonidine (or placebo in a double-blind cross-over design with a wash-out period of 7 days). The upright position was maintained for 1 h and a new blood sample taken. Heart rate and blood pressure were determined just before each blood collection.

Another protocol was performed to study the effects of α_2-adrenoceptor blockade on plasma NEFA and catecholamine concentrations during baroreceptor inactivation. The subjects were positioned in the supine position. Thirty min later, they ingested yohimbine (0.2 mg kg^{-1}) or placebo (in a double-blind cross-over design with a wash-out period of 7 days). A blood sample was taken after 1 h supine, afterwards, the subjects stood up and blood samples were taken 5 and 15 min later. Heart rate and blood pressure were determined just before each blood collection.

Plasma determinations

Plasma glucose and non-esterified fatty acids were determined with a glucose oxidase method and by the Wako enzymatic method respectively. For noradrenaline and adrenaline determinations, blood was collected on lithium heparin with 10 mmol l^{-1} sodium metabisulphite and centrifuged for 10 min at 4000 *g* at 0° C; the plasma was stored at −80° C. Catecholamines were selectively isolated from the plasma sample by adsorption on activated alumina, then eluted with 0.1 mol l^{-1} perchloric acid. Dihydrobenzylamine was used as internal standard to monitor recovery from the extraction step. Noradrenaline and adrenaline were assayed by high pressure liquid chromatography using electrochemical (amperometric) detection (Waters h.p.l.c. system) as previously described [16]. All the samples from each subject were analysed at the same time.

Chemicals

Yohimbine chlorhydrate® pills and clonidine (Catapressan®) pills were kindly given by Houdé Laboratories (Paris, France) and Boehringer Ingelheim (Paris, France) respectively. Enzymes for non-esterified fatty acids (Wako commercial kit) and glucose determination were obtained from Biolyon (Lyon, France) and Biotrol (Paris, France) respectively. All other chemicals and organic solvents were of reagent grade.

Data analysis

Values are given as mean ± s.e. mean (standard error of the mean). Each subject served as his 0 time control. The mean ± s.e. mean was then determined and statistics were based on the comparison of the values for a given group at each time period. In these conditions data were analysed using the paired *t*-test. The comparison of the data obtained in the different protocols (clonidine *vs* clinostatism test and yohimbine *vs* placebo during orthostatism test) was performed using analysis of variance (ANOVA). Differences were considered significant when P was smaller than 0.05.

Results

Plasma NEFA and catecholamine concentrations during reduction of sympathetic nervous system

Table 1 depicts the effects of a 60 min rest in the supine position (clinostatism) on various endocrino-metabolic and cardiovascular parameters. Rest provoked a dramatic decrease in plasma noradrenaline concentrations whereas plasma NEFA concentrations weakly, but significantly, increased. The other biological parameters studied (plasma adrenaline and glucose concentrations) remained unchanged. Resting induced a reduction in heat rate with no change in blood pressure.

Table 2 reports the effect of oral administration of 150 μg clonidine (or placebo) in the subjects maintained for 30 min in upright position before clonidine administration. Analysis of variance of basal values (before clonidine or placebo administration) of all the parameters studied during clonidine *vs* placebo days were not different. One hour after clonidine, a significant decrease in plasma NEFA and noradrenaline concentrations and in systolic, diastolic blood pressure and heart rate was observed. The other parameters studied were unchanged (plasma adrenaline and glucose concentrations). The administration of placebo did not induce any modification of all the studied parameters.

Analysis of variance of the variation in plasma noradrenaline and NEFA concentrations obtained during clonidine and clinostatism test showed that decrease in plasma noradrenaline concentration was significantly more pronounced during clinostatism ($P < 0.05$).

Table 1 Effect of a 60 min period of rest (lying position) on plasma non-esterified fatty acid, catecholamine and glucose concentrations and on cardiovascular parameters in seven healthy volunteers

	Standing	*60 min supine*	
NEFA ($\mu mol\ l^{-1}$)	227 ± 18	278 ± 20	$P < 0.05$
Noradrenaline ($pg\ ml^{-1}$)	433 ± 49	169 ± 13	$P < 0.01$
Adrenaline ($pg\ ml^{-1}$)	51 ± 9	37 ± 6	NS
Glucose ($mmol\ l^{-1}$)	5.4 ± 0.1	5.3 ± 0.2	NS
SBP (mm Hg)	132 ± 8	126 ± 7	NS
DBP (mm Hg)	82 ± 5	80 ± 3	NS
Heart rate ($beats\ min^{-1}$)	81.7 ± 4.2	66.5 ± 3.7	$P < 0.05$

NEFA: non-esterified fatty acids, SBP: systolic blood pressure, DBP: diastolic blood pressure.

Table 2 Effect of clonidine (150 μg orally; CLO) or placebo (PLA) administration on plasma non-esterified fatty acid, catecholamine and glucose concentrations and on cardiovascular parameters in seven healthy volunteers

		Before	*After (60 min)*	
NEFA ($\mu mol\ l^{-1}$)	CLO	289 ± 36	190 ± 26	$P < 0.01$
	PLA	372 ± 49	393 ± 49	NS
Noradrenaline ($pg\ ml^{-1}$)	CLO	555 ± 80	432 ± 74	$P < 0.05$
	PLA	410 ± 54	426 ± 42	NS
Adrenaline ($pg\ ml^{-1}$)	CLO	74 ± 14	73 ± 18	NS
	PLA	83 ± 16	73 ± 18	NS
Glucose ($mmol\ l^{-1}$)	CLO	4.8 ± 0.2	4.8 ± 0.1	NS
	PLA	5.3 ± 0.2	5.5 ± 0.3	NS
SBP (mm Hg)	CLO	124 ± 3	111 ± 2	$P < 0.01$
	PLA	120 ± 3	118 ± 2	NS
DBP (mm Hg)	CLO	85 ± 3	77 ± 2	$P < 0.01$
	PLA	74 ± 4	74 ± 2	NS
Heart rate ($beats\ min^{-1}$)	CLO	80.1 ± 5.3	72.4 ± 3.1	$P < 0.05$
	PLA	73.2 ± 2.4	71.5 ± 2.3	NS

NEFA: non-esterified fatty acids, SBP: systolic blood pressure, DBP: diastolic blood pressure.

Effect of yohimbine on plasma NEFA and catecholamine concentrations in the supine position: effect of postural rise

In the supine position and during the double-blind study *vs* placebo, yohimbine administered by the oral route ($0.2\ mg\ kg^{-1}$) elicited an 80–100% increase in plasma noradrenaline concentrations ($P < 0.02$). Plasma NEFA concentration was also increased when compared with placebo administration (Figure 1). Cardiovascular parameters and plasma glucose and adrenaline concentrations remained unchanged after yohimbine administration (Table 3). In the placebo design, 5 and 15 min after taking the upright position, the plasma concentration of noradrenaline was increased by about 200 $pg\ ml^{-1}$ whereas plasma NEFA concentrations slightly, but significantly, decreased at time 5 min (218 ± 21 *vs* 278 ± 20 $\mu mol\ l^{-1}$; $P < 0.05$) and remained unchanged at time 15 min (Figure 1). This manoeuvre induced an increase in heart rate without changes in arterial blood pressure (Table 3). The modifications induced by yohimbine after standing were very different (Figure 1). The increase in plasma noradrenaline was very large (the mean increment in plasma noradrenaline was 620 $pg\ ml^{-1}$ *vs* 200 $pg\ ml^{-1}$ in the same condition after placebo). A significant increase in plasma NEFA concentration was observed after 5 and 15 min upright (when compared with the corresponding values after placebo administration) and after 15 min when compared with values measured in the supine position. Arterial blood presure was not modified and the heart rate increased in the same proportions as with placebo. Plasma glucose and adrenaline concentrations remained unchanged (Table 3).

Variance analysis of the data showed that whatever the situation (supine position or during postural change) plasma noradrenaline and NEFA concentrations were significantly higher after yohimbine administration.

Discussion

The change from the upright position to the supine position induces a large reduction in plasma noradrenaline concentrations whereas the plasma NEFA concentration slightly increases. Thus, a physiological

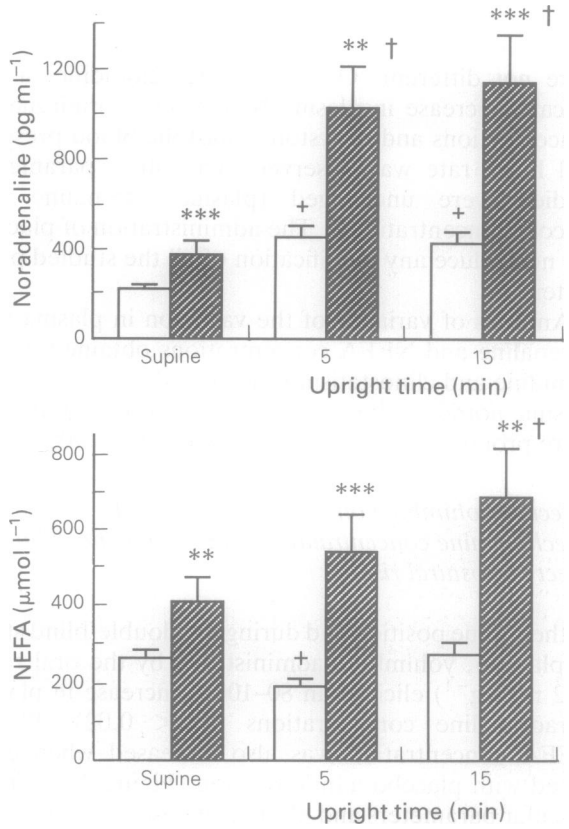

Figure 1 Effect of oral yohimbine administration (0.2 mg kg^{-1} orally) on plasma noradrenaline and non-esterified fatty acid concentrations. Yohimbine (or placebo) was administered 30 min after the subjects were placed in the supine position and a blood sample was taken 1 h later. Then, the subjects took the upright position and blood samples were taken 5 and 15 min later. NEFA: non-esterified fatty acid concentrations. □ placebo, ▨ yohimbine. $+P < 0.05$ when compared with supine placebo values using paired *t*-test. $\dagger P < 0.05$ when compared with supine yohimbine values paired *t*-test. $*P < 0.05$; $**P < 0.02$; $***P < 0.01$ when compared with corresponding values under placebo according to ANOVA analysis.

Table 3 Effect of oral yohimbine administration (0.2 mg kg^{-1}) on plasma glucose and adrenaline concentrations and on cardiovascular parameters in seven healthy volunteers in supine and upright position

	Supine	*Upright (5 min)*	*Upright (15 min)*
Glucose (mmol l^{-1})			
Placebo	5.3 ± 0.2	5.4 ± 0.1	5.4 ± 0.2
Yohimbine	5.1 ± 0.3	5.2 ± 0.3	5.1 ± 0.3
Adrenaline (pg ml^{-1})			
Placebo	25 ± 8	22 ± 7	27 ± 6
Yohimbine	22 ± 7	27 ± 8	24 ± 10
Systolic blood pressure (mm Hg)			
Placbo	126 ± 7	132 ± 8	132 ± 7
Yohimbine	127 ± 3	128 ± 5	130 ± 5
Diastolic blood pressure (mm Hg)			
Placebo	80 ± 3	85 ± 7	82 ± 5
Yohimbine	76 ± 4	81 ± 4	78 ± 6
Heart rate (beats min^{-1})			
Placebo	66.8 ± 3.2	75.3 ± 1.6	81.7 ± 4.2
		$P < 0.05$	$P < 0.02$
Yohimbine	63.5 ± 2.6	75.8 ± 3.1	75.6 ± 2.7
		$P < 0.02$	$P < 0.02$

Yohimbine (or placebo) was administered 30 min after the subjects were placed in the supine position and a blood sample was taken 1 h later. Then, the subjects stood up and blood samples were taken after 5 and 15 min.

reduction of sympathetic activity did not decrease plasma NEFA concentration. Oral clonidine significantly reduces plasma NEFA concentrations and, as previously demonstrated, also reduces plasma noradrenaline concentrations [17, 18]. The effect of the α_2-adrenoceptor agonist on plasma NEFA concentration can be due to its direct action on the antilipolytic α_2-adrenoceptors of adipose tissue and to a decrease of the effect of β-adrenoceptor-induced lipolysis due to the reduction of plasma noradrenaline concentration (indirect mechanism). Inferred from these two protocols, the results suggest that the effect of clonidine on plasma NEFA concentrations was rather attributable to a direct stimulation of the antilipolytic fat cell α_2-adrenoceptors than to the consequences of the decrease of the sympathetic nervous system activity. However, vascular mechanisms which could modify plasma NEFA concentrations should be considered. Clonidine, by acting on vascular α_2-adrenoceptors is able to reduce blood flow locally in adipose tissue. It has been shown that a decrease in blood flow in this tissue delayed glycerol and NEFA mobilization [19] and increased the re-utilisation of NEFA through enhancement of α-glycerophosphate formation [20].

A change from supine to the upright position is a classical method for the study of baroreflex inactivation. This manoeuvre induces an increase in heart rate and peripheral vasoconstriction without major changes in arterial blood pressure. Since the sympathetic responsiveness may be monitored by plasma catecholamines [21–23], the contribution of the sympathetic nervous system to these adaptations was demonstrated by the increase in plasma noradrenaline levels observed in the upright position (Figure 1). In agreement with earlier experiments [23], plasma noradrenaline concentrations and heart rate increased when the subjects stand up. Moreover, this increase in sympathetic tone was accompanied by a weak but significant decrease of plasma NEFA concentrations (after 5 min, Figure 1). According to previous data from Linde & Hjemdahl [24] it can be assumed that this response pattern is the consequence of a local vasoconstriction of sympathetic origin which fades within 15 min. Glycerol outflow, measured in venous blood, is initially impaired by the vasoconstriction (during the first 3 min of tilting) but pre-tilting outflow values were rapidly recovered when stimulation was maintained [24]. Moreover, a transient effect of sympathetic nerve stimulation on local circulation has also been observed in isolated animal adipose tissue [25].

Yohimbine administration elicited, in the supine position, a 70% increase in noradrenaline plasma levels. This result, in accordance with previous experiments [21, 26, 27], suggests that in the supine position,

the α_2-adrenoceptors located on the sympathetic nervous system tonically inhibit sympathetic outflow from nerve endings. Moreover, plasma NEFA concentration increases significantly (Figure 1).

Yohimbine administration dramatically modified responses to active postural changes (Table 3 and Figure 1). In the upright position we observed a large increase in plasma noradrenaline concentrations. This effect of yohimbine also suggests that α_2-adrenoceptors are important regulators of noradrenaline outflow in humans during changes from the supine to the upright position. After yohimbine, plasma NEFA concentrations were markedly increased when the subjects stood up. The effect of the drug can result from a direct blockade of the fat cell antilipolytic α_2-adrenoceptors and/or to enhanced β-adrenergic stimulation due to the increased sympathetic nerve activity. However, a local vasodilator effect of yohimbine in adipose tissue cannot be ignored. In fact, it is not possible to distinguish the various mechanisms occurring which are responsible for the NEFA mobilization after yohimbine. Fat cell α_2-adrenoceptor blockade is probably of importance since propranolol administration did not totally abolish the effect of yohimbine on NEFA mobilization in subjects maintained in the upright position [13, 14]. Keller *et al.* [11] also reported a transient increase in plasma NEFA and glycerol when noradrenaline was infused in man in association with phentolamine. The additive effect of phentolamine faded 40 min after the beginning of the infusion and the remaining lipolytic effect was abolished after propranolol administration. So, the increased concentration of plasma NEFA observed in the upright position is probably due both to a β-adrenergic stimulation and to the blockade of the α_2-adrenoceptors in adipose tissue. The vasodilator effect of yohimbine on NEFA output is rather difficult to evaluate but it could result in the increase of NEFA outflow from adipose tissue since the drug presumably attenuates locally the vasoconstrictor response to standing.

Plasma adrenaline concentrations were unmodified in all the protocols (Tables 1, 2 and 3); this suggests that only noradrenaline affects NEFA release from adipose tissue. This observation is in accordance with the experiments of Scheurink *et al.* [8, 9] who demonstrated that, whereas adrenaline directly affects glucose concentration and insulin release in exercising rats, only noradrenaline released from peripheral sympathetic nerves acts as the lipolytic agent.

Although yohimbine strongly raised plasma noradrenaline, no changes in arterial blood presssure were noticed either in the supine position or after standing. Moreover, the increase in heart rate induced by postural rise was not reinforced after yohimbine when compared with placebo (Table 3). These data could be explained by both the blockade of vascular α_2- and probably of α_1-adrenoceptors (the α_2/α_1-selectivity ratio of yohimbine is low and at the dose used the drug may also interact with vascular α_1-adrenoceptors). Concerning heart rate, a distinct possibility is that yohimbine may strengthen the vagal action on the heart rate (presumably by blocking the α_2-adrenoceptors mediating cholinergic transmission). This hypothesis was deduced from the fact that yohimbine increases salivary secretion in the dog and in humans [28, 29] through chorda tympani activation [28] and activates colonic motility in the dog through vagus nerve stimulation [30].

The present study demonstrates that physiological variations of sympathetic nervous system activity (induced through baroreflex activation or inactivation) weakly modify circulating NEFA concentration. In fact, plasma NEFA concentrations is crude measurement of events in adipose tissue and vasoactive processes are probably of major importance for NEFA output from adipose tissue. The data obtained during the changes from supine to upright position in plasma NEFA and catecholamine concentrations in the absence or presence of α_2-adrenoceptor blocking agent suggest that *in vivo*, plasma NEFA concentration is the result of the stimulation of antagonistic α_2- and β-adrenoceptors in adipose tissue. This protocol associating physiological sympathetic activation with α_2-adrenoceptor blockade seems to be a valuable method to investigate the relation between the activity of the sympathetic nervous system and the *in vivo* release of NEFA from adipose tissue in various physiopathological situations such as obesity, age or physical training.

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