The lipolytic effect of β_1 - and β_2 -adrenoceptor activation in healthy human volunteers

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- 1 We investigated the effect of activation β_1 - and β_2 -adrenoceptors on the process of lipolysis in human volunteers. Ten male subjects underwent a single-blind randomized cross-over trial using infusions of terbutaline (a specific β_2 -adrenoceptor agonist), xamoterol (a partial β_1 -agonist with β_2 -adrenoceptor blocking activity) and saline (placebo control). The effect of these infusions on plasma potassium, glucose, free fatty acids (FFA) (total and individual) and insulin levels was studied.
- 2 Terbutaline infusion induced a significant rise in plasma glucose and a fall in plasma potassium in keeping with its β_2 -adrenoceptor stimulant properties. Xamoterol infusion had no significant effect on these values. Terbutaline infusion caused a greater rise in total and individual FFA than xamoterol, but both effects were significantly different from placebo.
- 3 The possible reasons for these results and their implications on the β -adrenergic control of lipolysis are discussed.

Introduction

Lipolysis, the mobilization of free fatty acids (FFA) by hydrolysis of triacylglycerol stored in adipose tissue, occurs during periods of starvation, stress and exercise in response to specific neural and hormonal stimuli (Himms-Hagen, 1970; Smith, 1983). Each fat cell (adipocyte) contains a large triglyceride (TG) droplet in which FAs are linked to glycerol: these are released during lipolysis and catecholamines are the most important hormones regulating this process in man. Adipocyte membranes contain several adrenoceptor types: β -adrenoceptors, whose stimulation leads to adenylate cyclase activation, cyclic AMP formation and lipolysis; α_2 -adrenoceptors which cause the opposite effect (Kather, 1980; Wright, 1981); α_1 -adrenoceptors which mediate phospholipid turnover (Motulsky & Insel, 1982).

The plasma concentrations of free fatty acids (FFA) are potentially important. Low concentrations, caused by impaired release of FFA, may be a reason for reduced exercise capacity in patients on β -adrenoceptor blocking drugs (Lundborg *et al.*, 1981). High concentrations are associated with myocardial dysfunction and ventricular arrhythmias (Henderson *et al.*, 1970; Kurien & Oliver, 1970). β_2 -adrenoceptor agonist drugs cause elevation of FFA concentration and this may be relevant to the unacceptably high mortality among asthma sufferers which some attribute to excessive use of β_2 -adrenoceptor agonists (Abbott, 1988).

The nature of the β_2 -adrenoceptor subtype involved in lipolysis varies between species and it is still uncertain which human adipocyte β -adrenoceptor, β_1 or β_2 , is more important in the process of lipolysis. We chose to investigate this question by comparing the effects of terbutaline, a specific β_2 -adrenoceptor agonist, with xamoterol, a β_1 -adrenoceptor agonist with about 43% of the intrinsic sympathomimetic activity of isoprenaline and β_2 -adrenoceptor blocking properties (Nuttall & Snow, 1982). Xamoterol was chosen because its partial agonist effect is β_1 -selective. Dobutamine is less β_1 specific and has significant α_1 adrenoceptor action, noradrenaline also has substantial α activity and the β_1 -selectivity of prenalterol is disputed (Malta *et al.*, 1985).

Method

Ten healthy male volunteers, mean age 22.6 years (range 19–33 years) participated in this single-blind randomized cross over study after their fitness had been established by physical examination and full haematological and biochemical screening. They all gave fully informed consent and the study had been approved by the Central Birmingham Healthy Authority Ethics Committee.

Each volunteer attended our Clinical Investigation

Unit on 3 separate mornings at least 1 week apart, having fasted overnight. Intravenous cannulae were inserted into a forearm vein of each arm and there followed a 60 min rest period during which two blood samples were taken for baseline biochemical values and pulse and blood pressure were also measured. Then each volunteer received one of three treatments:

A xamoterol

75 $\mu\text{g kg}^{-1}$ as bolus intravenous injection in 0.9% saline followed by infusion of 80 $\mu\text{g kg}^{-1} \text{h}^{-1}$ for 1 h.

B terbutaline

Infusion of 6 $\mu\text{g kg}^{-1} \text{h}^{-1}$ in 0.9% saline for 1 h.

C placebo

0.9% saline 50 ml for 1 h.

The doses chosen for xamoterol bolus and infusion were determined by pharmacokinetic studies performed by Bastain *et al.* (1988) and Marlow *et al.* (1990).

Blood was withdrawn at 15 min intervals into lithium heparin tubes and centrifuged immediately. The plasma was then stored at -70°C until potassium was assayed by flame photometry and FFAs by a gas liquid chromatographic method (Ko & Roger, 1974). In this, the extraction solution was propan-2-ol in hexane (14:6 ratio), 0.015 M sulphuric acid was used, methanoic HCl replaced *N*, *N'*-carbonyldiimidazole and hexane replaced triethylamine. Plasma glucose was assayed by a glucose oxidase method after collections into oxalate tubes. Insulin was measured by radioimmunoassay using a double antibody separation method. The plasma sample was incubated overnight at room temperature with guinea pig anti-insulin antiserum and a trace amount of iodinated insulin. Separation was achieved by addition of a single reagent incorporating polyethylene glycol, donkey anti-guinea pig serum and carrier serum. The sample was centrifuged after 30 min and the supernatant counted. Xamoterol plasma concentrations were measured using a radioimmunoassay procedure described by Bastain *et al.* (1988). Blood pressure and pulse rate were measured using an automated device (Dynamap Critikon). The subjects rested supine throughout the study and measurements were continued for 2 h following the end of the infusion.

Statistics

Changes in biochemical and haemodynamic variables from baseline for all 10 volunteers were analysed using a random effects ANOVA. A *P* value of <0.05 was taken as significant.

Results

The mean of the two preinfusion values for all variables was taken as baseline. There was no significant difference between treatment groups for these. Figure 1 shows mean changes from baseline values for glucose and potassium (with s.e. mean and levels of significance). There was a significant fall in plasma potassium ($P < 0.001$) and rise in plasma glucose ($P < 0.001$) during terbutaline infusion which did not occur during xamoterol

Figure 1 Change in glucose and potassium from baseline during infusions of terbutaline ($\square$), xamoterol ($\blacklozenge$) (mmol l^{-1}) and placebo ($\blacksquare$) (mean $\pm$ s.e. mean).

Figure 2 Mean $\pm$ s.e. mean xamoterol concentrations (ng ml^{-1}) during xamoterol infusion.

or saline. Terbutaline also caused a rise in heart rate ($P < 0.001$) and fall in diastolic pressure ($P < 0.001$) whereas xamoterol led to a rise in systolic pressure ($P < 0.009$) and heart rate ($P < 0.001$) (Table 1). This confirms their respective β_2 - and β_1 -agonist effects.

FFA levels increased during terbutaline and xamoterol infusions (both $P < 0.001$) but the effect of terbutaline was greater than that of xamoterol (Table 2). The effects of these infusions on plasma insulin are shown in Table 3. A significant rise in plasma insulin occurred during terbutaline infusion ($P < 0.001$) and xamoterol infusion ($P = 0.001$) but the former was far greater. Figure 2 shows mean xamoterol plasma concentrations.

Table 1 Changes in haemodynamic data (and 95% confidence intervals)

Time (min)	Terbutaline	Xamoterol	Saline
<i>Pulse (beats min⁻¹)*</i>			
Baseline	56.6 (52.4 – 60.8)	56.7 (51.1 – 62.2)	58.4 (54.2 – 62.7)
75	11.6 (7.2 – 16.0)	8.1 (4.2 – 11.9)	-0.9 (-3.5 – 1.6)
90	15.8 (11.9 – 19.8)	7.8 (4.4 – 11.2)	-0.25 (-3.7 – 3.2)
105	20.1 (16.2 – 25.1)	11.3 (5.1 – 17.6)	0.25 (-4.6 – 5.1)
120	22.75 (18.6 – 26.9)	8.4 (3.0 – 13.8)	0.5 (-2.0 – 3.0)
150	17.8 (12.3 – 23.4)	8.1 (4.6 – 11.5)	-0.7 (-3.0 – 1.6)
180	9.8 (5.9 – 13.6)	9.3 (5.3 – 13.2)	-0.7 (-2.8 – 4.1)
<i>Systolic BP (mmHg)**</i>			
Baseline	122.75 (116.6 – 128.9)	117.3 (112.2 – 122.5)	122.2 (115.3 – 129.2)
75	2.75 (-2.1 – 7.6)	17.8 (14.5 – 21.2)	-1.8 (-6.6 – 2.9)
90	2.7 (-2.8 – 8.2)	17.0 (11.5 – 22.5)	-2.3 (-6.5 – 1.8)
105	1.3 (-4.3 – 7.0)	21.4 (15.5 – 27.3)	-4.4 (-9.3 – 0.5)
120	1.2 (-3.8 – 6.1)	17.5 (8.7 – 26.3)	-2.25 (-6.7 – 2.2)
150	0.2 (-6.7 – 7.0)	15.4 (13.3 – 17.5)	0.7 (-3.7 – 5.0)
180	2.0 (-2.0 – 6.0)	15.75 (11.1 – 20.4)	3.7 (0.1 – 7.2)
<i>Diastolic BP (mmHg)***</i>			
Baseline	67.6 (63.1 – 72.1)	62.7 (59.7 – 65.6)	64.6 (59.7 – 69.4)
75	-4.7 (-8.8 – 0.5)	5.3 (3.1 – 7.5)	-0.6 (-3.7 – 2.5)
90	-5.2 (-9.1 – -1.4)	2.0 (-1.9 – 5.9)	-1.1 (-4.9 – 2.7)
105	-6.6 (-10.6 – -2.6)	4.0 (-0.4 – 8.6)	-0.4 (-3.5 – 2.7)
120	-8.8 (-14.3 – -3.3)	4.6 (-1.7 – 10.9)	1.8 (-1.7 – 5.4)
150	-8.0 (-13.0 – -3.0)	4.5 (0.1 – 8.9)	0.25 (-2.5 – 3.0)
180	-4.0 (-8.6 – 0.4)	6.0 (1.3 – 10.6)	0.9 (-2.9 – 4.8)

* $P < 0.001$ for terbutaline and xamoterol compared with saline at all times.

** $P < 0.052$ for terbutaline and $P = 0.009$ for xamoterol compared with saline at all times.

*** $P < 0.001$ for terbutaline and $P = 0.058$ for xamoterol compared with saline at all times.

Table 2 Changes in total free fatty acids and glucose (mean and confidence intervals, mmol l⁻¹)

Time (min)	Terbutaline	Xamoterol	Saline
<i>Free fatty acids (mmol l⁻¹)*</i>			
Baseline	0.365 (0.32 – 0.41)	0.382 (0.34 – 0.425)	0.374 (0.31 – 0.44)
75	0.355 (0.24 – 0.47)	0.239 (0.13 – 0.35)	0.034 (0 – 0.07)
90	0.440 (0.34 – 0.53)	0.265 (0.18 – 0.35)	0.042 (0 – 0.09)
105	0.432 (0.32 – 0.54)	0.173 (0.10 – 0.24)	0.037 (0 – 0.08)
120	0.359 (0.23 – 0.47)	0.187 (0.1 – 0.28)	0.052 (0 – 0.10)
150	0.123 (0.02 – 0.22)	0.160 (0.07 – 0.25)	0.057 (0 – 0.12)
180	0.062 (-0.04 – 0.16)	0.177 (0.06 – 0.29)	0.078 (0 – 0.15)
<i>Glucose (mmol l⁻¹)</i>			
Baseline	5.06 (4.79 – 5.33)	4.95 (4.69 – 5.21)	5.0 (4.78 – 5.21)
75	0.33 (0.20 – 0.46)	0.04 (-0.05 – 0.14)	0.22 (-0.08 – 0.51)
90	0.69 (0.48 – 0.90)	-0.025 (-0.15 – 0.10)	0.01 (-0.10 – 0.12)
105	1.00 (0.66 – 1.34)	-0.05 (-0.15 – 0.20)	0.02 (-0.17 – 0.20)
120	1.14 (0.81 – 1.47)	0.02 (-0.15 – 0.19)	0.025 (-0.16 – 0.21)
150	0.875 (0.53 – 1.22)	0.01 (-0.20 – 0.22)	0.01 (-0.19 – 0.21)
180	0.62 (0.32 – 0.91)	0.18 (-0.02 – 0.39)	0.05 (-0.09 – 0.19)

* $P < 0.0001$ for terbutaline and $P = 0.001$ for xamoterol compared with saline at all times.

** $P = 0.001$ for terbutaline and $P = 0.52$ for xamoterol compared with saline at all times.

Table 3 Plasma insulin (μ l l⁻¹)

Time (min)	Terbutaline	Xamoterol	Saline
Baseline	32.02 (17.7 – 46.3)	30.37 (22.86 – 37.89)	32.6 (26.23 – 38.97)
90	54.04 ** (36.94 – 71.15)	8.00 * (1.71 – 14.29)	-4.3 (-7.61 – 1.01)

** $P < 0.001$ when compared with saline

* $P = 0.001$.

Discussion

Adipocyte membrane adrenoceptors have been studied in a number of ways. Firstly, *in vitro* binding of radioactive ligands, whether agonists or antagonists, demonstrate the presence of α - or β -adrenoceptors and define their type. For instance, Engfelt (1982) incubated fat cells from adipose tissue of surgical patients

with (-)-[³H]-dihydroalprenolol and demonstrated a homogenous population of receptors, 80% (about 400,000 per cell) of which were β_1 in type. Using three non-selective radioligands and highly selective β_1 - and β_2 -adrenoceptor antagonists, Mauriege *et al.* (1988) studied membranes of abdominal subcutaneous tissue adipocytes from obese premenopausal women. β_1 - and β_2 -adrenoceptors were present in the ratio of 40:60. However these binding studies provide no information on the role of these receptor subtypes.

Secondly, the function of these adrenergic receptors has been studied by direct stimulation *in vivo* and *in vitro* and the effect of adrenergic blocking drugs has also been examined. Goldberg *et al.* (1975) gave bolus doses of intravenous salbutamol to nine male volunteers before and after treatment with oral practolol (β_1 -adrenoceptor antagonist). After each dose of salbutamol FFA levels rose swiftly to reach a peak at 4–5 min. Oral practolol did not prevent this, thus suggesting β_2 -adrenergic control. Ronn (1990) administered three different intravenous doses of prenalterol, a relatively selective β_1 -adrenoceptor agonist, to 10 healthy volunteers. A moderate, but non dose dependent, rise in FFA and glycerol occurred. This absence of dose relationship could reflect increased utilization of FFA (heart rate also increased) or it may be that prenalterol is not a pure β_1 -adrenoceptor agonist at adipocyte adrenoceptors.

Thirdly, the influence of other factors on lipolysis e.g. fasting, exercise have been considered in man and animals. Lundborg *et al.* (1981) showed that exercise induced lipolysis was inhibited by propranolol and to a lesser extent by metoprolol. This suggested that lipolysis was a function of β_1 - and β_2 -adrenoceptor stimulation. Koch *et al.* (1981) also investigated exercise induced lipolysis in hypertensive men and demonstrated that acebutolol and pindolol had comparable effects, supporting the involvement of both β_1 - and β_2 -adrenoceptor. Finally, Hall *et al.* (1987) compared the effects of β_1 -adrenoceptor blockers on FFA release during and after submaximal exercise in fit volunteers. This was inhibited more by pindolol and propranolol than by metoprolol, suggesting β_2 -adrenoceptors have a more important role in exercise induced lipolysis.

In the present study terbutaline behaved as a relatively selective β_2 -adrenoceptor agonist, increasing plasma glucose and insulin, lowering plasma potassium and diastolic pressure but not affecting systolic pressure. In comparison, xamoterol behaved pharma-

cologically as a relatively selective β_1 -adrenoceptor agonist, elevating systolic pressure and heart rate but not significantly altering other parameters. Both drugs caused a rise in FFA but the response to terbutaline was greater than that to xamoterol. This could be explained in a number of ways.

Firstly, adrenoceptor mediated lipolysis could be predominantly effected through β_2 -adrenoceptors. Secondly, the doses used may not have been comparable: the β_1 -stimulation by xamoterol may have been submaximal. Jennings *et al.* (1984) study showed that a plasma concentration of 215 $\mu\text{g ml}^{-1}$ xamoterol led to a maximal FFA response. The levels measured in this study were lower, but since the effect of the drug is proportional to log plasma concentration, our values would have achieved near maximal effect.

A third explanation for the difference between the two infusions could be that the effects of xamoterol were counteracted by another process. This possibility has been considered but there is no evidence for it.

Lastly it may be that adipocyte adrenoceptors do not actually behave like classical β_1 and β_2 -adrenoceptors. In mice insulin secretion is controlled by a β -adrenoceptor which cannot be classified as β_1 - or β_2 (Wilson *et al.*, 1984). Such a receptor, called β_3 , has also been suggested for rat adipocyte (Tan & Curtis-Prior, 1983). It could be that human adipocyte adrenoceptors behave similarly and that they have some β_1 and some β_2 properties, rather than there being a mixed population of receptors.

We have shown that terbutaline and xamoterol infusions produced increases in plasma FFA in healthy volunteers and suggest that this provides further evidence for lipolysis being a combined β_1 - and β_2 -adrenoceptor mediated process. It is difficult to determine the relative importance of β_1 and β_2 -adrenoceptor stimulation of lipolysis as this would require administration of exactly comparable amounts of the two pharmacological agents. However they have no other shared effects by which to assess this. The greater rise in FFA with terbutaline may suggest a more important role for β_2 -adrenoceptor in lipolysis.

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