Effects of terbutaline on basal thermogenesis of human skeletal muscle and Na-K pump after 1 week of oral use—a placebo controlled comparison with propranolol

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- 1 A double-blind placebo-controlled study was conducted on the effects of oral terbutaline (β_2 -adrenoceptor agonist) and propranolol ($\beta_1\beta_2$ -adrenoceptor antagonist) on basal heat production of skeletal muscle, measured *ex vivo* by direct microcalorimetry. Terbutaline slow-release 7.5 mg, propranolol 80 mg, and matching placebo were randomly administered twice daily for 1 week to 15 healthy males, using a cross-over design.
- 2 Resting heat production in biopsied vastus lateralis was lowered by median 27% ($P < 0.01$) after terbutaline medication as compared with placebo. The cause of this hypometabolism at the cellular level is obscure but may possibly be explained by desensitization of β_2 -receptors.
- 3 Propranolol decreased the metabolic rate by 17% ($P > 0.3$); this might imply that the sympathetic nervous system is playing only a minor role in the regulation of basal metabolic rate in muscle, or that up-regulation of β -receptors had influenced the decline.
- 4 The muscle utilized about 6% of its total energy for the Na-K pump as assessed after inhibition by ouabain.
- 5 Serum potassium was significantly lowered by terbutaline and slightly increased by propranolol with no relationship between changes in extracellular levels and muscle content of potassium under resting conditions. Energy values for the Na-K pump in muscle after 1 week of terbutaline or propranolol medication were similar to placebo. The results are not consistent with the hypothesis that decreased serum potassium during continuous β_2 -adrenoceptor agonist treatment is due to a chronically activated Na-K pump, at least not in resting muscle.

Keywords β -adrenoceptors electrolytes Na-K pump potassium
propranolol skeletal muscle terbutaline thermogenesis

Introduction

Physiological processes involved in the control of thermogenesis are usually studied by sympathetic activators and inhibitors. Oxygen consumption measurements in man have indicated that striated muscles at rest may be an important site of thermogenesis induced by sympathomimetic agents (ephedrine, noradrenaline, adrenaline), i.e. 'facultative' thermogenesis, or heat produced in excess of what is required to maintain the basal state (Astrup *et al.*, 1985; Lundholm & Svedmyr, 1965). Documentation is however sparse concerning the role of catecholamines in human muscle thermogenesis (Astrup, 1986; Lundholm *et al.*, 1966). Using microcalorimetry

we demonstrated a suppressed thermogenic response in human skeletal muscle after non-selective oral β -adrenoceptor blockade (Fagher *et al.*, 1986a), and the results seemed to reflect reliably the *in vivo* conditions (Fagher, 1988); results were achieved after performance of light physical work and did not represent the true basal state.

Very few metabolic studies have been performed in humans during chronic administration of selective β_2 -adrenoceptor agonists (Acheson *et al.*, 1988; Scheidegger *et al.*, 1984). In animals raised energy intake and weight gain were reported for fenoterol and clenbuterol (Dalrymple *et al.*, 1983; Emery *et al.*, 1984). However

the induced anabolic effect on striated rat muscle seems to have a complex pharmacology (Reeds *et al.*, 1988; Yang & McElligott, 1989) and the result obtained with clenbuterol was not demonstrated with other selective β_2 -adrenoceptor agonists like terbutaline and salbutamol (Reeds *et al.*, 1988); furthermore it is unclear at present whether the effect is direct or indirect. New dimensions have also been added recently to β -receptor classification (Yang & McElligott, 1989).

The present investigation was performed to evaluate whether basal heat production is influenced by continuous oral medication with a β_2 -adrenoceptor agonist, terbutaline, the effect being compared with placebo and the non-selective β -adrenoceptor antagonist propranolol. Biopsied human skeletal muscle was studied by direct microcalorimetric technique. Secondly, as we still lack a complete understanding of the mechanisms by which potassium changes can develop in response to β -adrenoceptor agonists or β -adrenoceptor antagonists (Gullestad *et al.*, 1991), an evaluation was made of the activity of the Na-K-pump after ouabain suppression in order to see if there is in resting muscle a chronic stimulatory or inhibitory effect after medication. Results were related to serum drug levels and electrolyte values in the intra- and extracellular space.

Methods

Experimental design

The study had a double-blind, cross-over design. Propranolol 80 mg twice daily (ICI, Macclesfield, U.K.), terbutaline slow release 7.5 mg twice daily (Astra-Draco, Sweden), and placebo were randomly administered for 7 days to 15 healthy non-smoking males, using a double-dummy technique. Placebo tablets of similar shape and colour were used. Subjects were 20–50 years old with a median age of 26 years; their mean body weight was 78 (s.d. 11) kg and body height 181 (s.d. 8) cm. Muscle biopsies and blood samples were taken after 7 days on each drug. 'Washout' periods were incorporated with at least 2 weeks separating the study periods. The subjects were encouraged not to change their physical activities and dietary habits during the study. They were informed of the aim of the study and possible risks of the investigation and gave their consent. No other medication was allowed. The study was approved by the Lund University Ethical Research Committee.

Calorimetry

The microcalorimeter used in the present work was of the thermopile heat-conduction type, arranged as a twin instrument with one of the units containing the reactive system and the other containing the reference ampoule with water (Wadsö, 1974). Measurements were performed at 37°C. A 0.7 cm³ flow-through vessel for liquid perfusion was used. A good baseline stability, established with the vessel charged with water, was obtained, < 2 μ W during 12 h. The voltage output was amplified with a Keithley 150B Microvoltmeter, 10 μ V range; calibration

was carried out electrically. The calorimetric vessel was thermostated in two consecutive heat exchange positions before reaching the thermopile zone after about 15–20 min. The perfusion medium (see below) was introduced very close to the resting muscle samples by the use of a peristaltic pump (LKB Perpex) in order to keep constant pH; perfusion rate was 5 cm³ h⁻¹. Oxygen was bubbled through the medium outside the calorimeter. Perfusion started within 38 min after biopsy and lasted approximately 4 h. During the experimental period, pH in the effluent was measured intermittently by a radiometer type G297/G7 capillary electrode; a very slight decrease in pH, on average 0.04, was observed. The muscle heat production rate as calculated from the power-time curves refers to the second hour after the start of the calorimetric measurements and is expressed in units of mW g⁻¹ wet muscle (Fagher, 1988). The coefficient of variation for the method is 4.2% (Fagher *et al.*, 1986b).

Muscle and blood sample preparation

After an overnight fast for 12 h, muscle biopsies and venous blood samples were taken at 09.00 h, 2.5 h after drug intake and after the subjects had been resting for at least 45 min. Muscle samples were taken from the middle portion of the vastus lateralis, at a depth of about 3–4 cm, using a 5 mm ($\varnothing$) needle (Bergström, 1975) (Stille, Sweden). Lignocaine with no adrenaline was used for anaesthesia of the subcutaneous tissue, down to the fascia, without infiltrating the muscle.

Muscle specimens were at first handled in ice-chilled Krebs-Ringer-phosphate (KRP) buffer (values in mol dm⁻³): 0.12 NaCl, 0.005 KCl, 0.001 CaCl₂, 0.016 Na₂HPO₄, 0.001 MgSO₄, pH 7.4, supplemented with 0.00825 glucose. They were also exposed to insulin (Actrapid[®] Novo, Denmark) in a final concentration of 0.1 unit cm⁻³ buffer, until the calorimetric experiment started (Fagher *et al.*, 1986b). Fibre bundles with a maximum thickness of 0.5–0.6 mm were teased away. According to data on solubility and diffusiveness of oxygen at 37°C (Kleiber, 1961) it was calculated that this muscle thickness allowed adequate oxygenation. The specimens were freed from visible connective tissue and fat, carefully blotted on filter paper and thereafter quickly weighed just before the calorimetric measurement; mean muscle weight was 37.4 mg (s.d. 4.5) in the experiments.

Venous blood was collected in Serum Separation Tubes (Becton & Dickinson), for analyses of potassium, magnesium and sodium in serum after the red blood cells had been separated by centrifuging at 300 g for 10 min. Venous blood for drug analysis was collected in Venoject tubes without additive and centrifuged; the serum samples were stored at -20°C until analyses of propranolol (Pritchard *et al.*, 1979) and terbutaline (Borgström *et al.*, 1989) were made by h.p.l.c. and by GCMS, respectively; metabolites of propranolol were not analyzed. Mean serum concentration of propranolol was 334 (s.d. 144) nmol dm⁻³ and that of terbutaline 15.3 (s.d. 5.8) nmol dm⁻³.

Muscle electrolytes

Parts of the muscle samples were immediately frozen after biopsy and stored at -20°C. The tissue was later

dried at 110°C overnight. Neutral fat was extracted with petroleum ether in a quartz glass tube. After another 3 h at 110°C, the fat-free dry solid weight was measured. Electrolytes were extracted by 0.5 cm³ nitric acid (1 mol dm⁻³) in 1% LaCl₃. The samples were then diluted with a solution consisting of LaCl₃ (10,000 ppm) and CsCl (1,000 ppm) in 1% nitric acid. Analyses of K⁺ and Mg⁺⁺ contents in muscle, expressed as mmol 100 g⁻¹ of fat-free dry solid, were performed with atomic absorption spectroscopy (Sjögren *et al.*, 1987).

Energy expenditure of the Na-K pump

Ouabain octahydrate (10⁻⁴ mol dm⁻³) was added to the perfusion medium after incubation of the muscle samples for 2 h in the calorimetric vessel (Fagher *et al.*, 1987). The ouabain-induced depression of heat production (ΔP) was also calculated as a percentage of the total resting heat production.

In vitro drug experiments

In a series of calorimetric experiments with muscle specimens (vastus lateralis) from two healthy males, aged 50 and 54 years, terbutaline sulphate (Astra, Sweden) was added *in vitro* to the KRP buffer, using glucose as substrate and both with and without insulin in the medium. Final terbutaline concentrations were 0.02, 0.2, 2, and 60 μ mol dm⁻³. Similarly, propranolol hydrochloride (ICI-Diagnostika, Falkenberg, Sweden) was added to the calorimeter in two experiments in a final concentration of 0.3 μ mol dm⁻³. A 4-channel microcalorimeter (Bio-Activity Monitor 2277, LKB, Bromma, Sweden) was used, with a 3 cm³ perfusion vessel where the muscle samples were contained in a cage acting as a stirrer, the perfusion rate being 20 cm³ h⁻¹ and the stirring rate 24 rev min⁻¹ (Fagher *et al.*, 1986b).

Statistics

Means and standard deviations (s.d.) are given; also median values and 95% confidence intervals are used. The estimation of sample size was based on the assumption that a difference of at least 25% of the mean (0.7 mW g⁻¹, s.d. 0.1) of normal muscle heat production rate (Fagher *et al.*, 1986b) was to be detected. With an α -error of 2% (due to three comparisons of the means) and a power of 80%, the study should include at least seven subjects. As there was no knowledge of the influence of terbutaline on muscle heat production, the sample size was increased to include 15 volunteers. The *a posteriori* calculation of the actual power of the study from the observed intergroup differences gave a power of 70 to 85%, depending on the number (3 or 1) of statistical calculations (Machin & Campbell, 1987). Differences in muscle heat production were tested by the Wilcoxon test for paired data. The significance was further defined by calculating the 95% confidence interval for the difference, using a nonparametric, paired design (Anderson *et al.*, 1982). Other intergroup differences have been tested by analyses of variance (ANOVA). Spearman's rank test was used for calculating the correlation coefficients (r_s).

Results

Heat production ex vivo (Table 1)

Oral propranolol and terbutaline lowered the median resting heat production rate of skeletal muscle in comparison with placebo by 17% (nonsignificant, $P > 0.3$) and 27% ($P < 0.01$), respectively. The confidence interval for the difference between placebo and terbutaline values was 0.06 to 0.36 mW g⁻¹ muscle. Significant drug concentration-effect relationships were not apparent ($r_s = -0.42$ and -0.38 , respectively).

Table 1 Comparative effects of 7 days treatment with propranolol (*Pr.*) 80 mg twice daily, and terbutaline (*T*) slow-release 7.5 mg twice daily on basal heat production (P = power) of muscle (vastus lateralis), and electrolytes in serum and muscle in 15 healthy male subjects

	Placebo	Propranolol	Terbutaline	Significance of difference <i>Pr.-T.</i>
<i>Heat production rate (mW g⁻¹ wet weight)</i>				Wilcoxon
<i>P</i>	0.78 (0.56–0.99)	0.64 (0.51–0.78)	0.59 (0.45–0.72) ^a	NS
<i>Electrolytes</i>				
<i>serum (mmol dm⁻³)</i>				ANOVA
K	4.1 (0.2)	4.3 (0.4)	3.8 (0.4) ^b	< 0.001
Na	139.6 (2.1)	139.7 (2.9)	140.5 (1.1)	NS
Mg	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)	NS
<i>muscle (mmol 100 g⁻¹ of fat-free dry solid)</i>				
K	45.6 (3.5)	45.2 (2.4)	47.2 (2.1)	NS
Mg	4.6 (0.4)	4.5 (0.3)	4.8 (0.3)	NS
ΔK (after ouabain)	-2.1 (5.2)	-2.2 (3.7)	-5.0 (2.4)	NS

Values are means with s.d. or 95% confidence intervals within parentheses.

Significance vs placebo: ^a = $P < 0.01$, ^b = $P < 0.05$.

Table 2 Comparative effects on haemodynamic variables

	Placebo	Propranolol	Terbutaline	Significance of difference Pr.-T. ANOVA
Heart rate (beats min ⁻¹)	58 (11)	50 (6) ^b	69 (9) ^a	< 0.001
Systolic blood pressure (mm Hg)	122 (6)	119 (9)	125 (6)	< 0.05
Mean blood pressure (mm Hg)	88 (5)	87 (6)	88 (6)	NS

Values are means (s.d.).

Significance vs placebo: ^a = $P < 0.01$, ^b = $P < 0.05$.

The pharmacological influence of the drugs was also exhibited by changes in haemodynamic variables at the end of the study (Table 2). Terbutaline caused a significant increase of heart rate but no change in blood pressure.

Irrespective of treatment, incubation with ouabain decreased thermogenesis consistently in muscle. Mean ΔP for muscle were after placebo -5.5% , after propranolol -6.4% , and after terbutaline treatment -5.1% .

Electrolytes (Table 1)

Compared with placebo a significant decrease in serum potassium by a median of 7% was seen after terbutaline treatment. Correspondingly, a slight but insignificant increase in muscle potassium was measured. The change in serum potassium was inversely correlated with the serum concentration of terbutaline, $r_s = -0.55$ ($P < 0.05$, $n = 14$; Figure 1), whereas no significant relationship ($r_s = 0.29$) was found with the propranolol concentration.

There was a minor nonsignificant increase in serum potassium after propranolol treatment (median 1%) and an essentially unchanged mean potassium content in muscle. However, individual changes in muscle potassium (above and below zero) were positively correlated with the serum levels of propranolol, $r_s = 0.77$ ($P < 0.025$). No such relationship was seen for terbutaline ($r_s = 0.24$). Alterations in muscle potassium from placebo values did not correlate with the corresponding changes in serum potassium, either after propranolol ($r_s = -0.20$) or terbutaline ($r_s = -0.0$).

In vitro drug experiments

Terbutaline and propranolol had no effect on heat production of biopsied muscle from two subjects when added *in vitro* in increasing concentrations. In contrast, more than a 50% decline in muscle heat production rate occurred after terbutaline had been given orally for 1 week to one of these subjects, who also took part in the main study.

Discussion

The metabolic actions of different β_2 -adrenoceptor agonists on striated muscle have not been extensively studied but seem not, as assessed in animal work, to be similar (Reeds *et al.*, 1988). Unexpectedly, in our system both the β -adrenoceptor agonist and the β -adrenoceptor antagonist appeared to reduce basal thermogenesis. The reason for a clear hypometabolism at the cellular level after 1 week of terbutaline medication is obscure and may possibly be explained by desensitization of β_2 -

Figure 1 Changes (Δ) in serum potassium in relation to the serum concentrations of terbutaline after 1 week of medication ($r_s = -0.55$, $P < 0.05$, $n = 14$).

receptors in muscle. In the perfusion system used, medication will be washed out, but since resensitization has a slow time-course (Martinsson *et al.*, 1987) the measured heat production rate is supposed to reflect the β -receptor function *in vivo*. As to the more modest decline in metabolic rate after propranolol, it is conceivable that an 'up-regulation' of the β -receptors might have interfered. New experiments must clarify that.

It is of note that in our previous study levels of endogenous adrenaline in a group of untreated hypertensives were inversely correlated with the heat production rates in skeletal muscle as measured *in vitro* (Fagher *et al.*, 1988). The mechanism might have been that the number of β -receptors in metabolically less active tissue is reduced with decreased binding of adrenaline.

Our present observations give no information on possible mechanisms of desensitization to the β -adrenoceptor agonist (see review, Davies *et al.*, 1984). It is, however, well-known that with continuous use of β_2 -adrenoceptor agonists a certain degree of tachyphylaxis or subsensitivity of β -responses will develop (Cheung *et al.*, 1992; Jenne, 1982; Martinsson *et al.*, 1987; O'Connor *et al.*, 1992; Weber *et al.*, 1982), although some conflicting results regarding the effects on the airway have been presented (Larsson *et al.*, 1977; Tashkin *et al.*, 1982). In guinea-pig skeletal muscle it was shown that treatment with terbutaline for 5 (Buur *et al.*, 1982) or 7 days (Elfellah & Reid, 1990) caused a desensitization of β -receptors, similar to adrenaline or isoprenaline (Elfellah & Reid, 1990). In the latter study, a significant effect was seen only when treatment was continued for 7 days but not when limited to 3 days. In humans,

comparison of chronic vs acute administration of terbutaline has demonstrated an inhibited metabolic response of plasma glucose concentrations and insulin secretion during continuous use, indicating the development of tolerance (Wager *et al.*, 1981). Acheson *et al.* (1988) found no significant change in basal metabolism after terbutaline in comparison with placebo and propranolol in a 2-week trial, although certain alterations in substrate utilization occurred. Scheidegger *et al.* (1984a,b) showed that basal whole-body respiratory exchange was slightly increased by 7% after 2 weeks of terbutaline treatment. Basal glucose oxidation was unchanged, whereas the isoprenaline-induced thermogenic response was blunted. It was suggested that β -receptor stimulation of thyroid hormones was partially responsible for this metabolic effect.

In the present experimental situation propranolol had a modest, nonsignificant effect on heat production from the muscle. The possibility should be considered that β -adrenoceptor blockade may not have been complete. However, the average plasma concentration of propranolol was higher than in our previous investigation in normal subjects (Fagher *et al.*, 1986a), in which a significantly lowered muscle heat production was achieved after 8 days of treatment. In that study muscle biopsies were taken after light physical exercise, which means at an increased sympathoadrenal activity. A more pronounced inhibitory response to an antiadrenergic drug is expected with higher sympathetic tone, and, in fact the sympathetic nervous system has been regarded previously as playing only a minor role in the regulation of basal metabolic rate. Regarding the possibility of 'up-regulation' of β -receptors during continuous blockade, it can be exemplified by our earlier findings in healthy subjects of a more pronounced decline in physical performance and heart rate immediately after the institution of treatment than after 4 days (Fagher *et al.*, 1985).

In previous studies propranolol seemed to have no effect on basal metabolic rate in humans, using oxygen consumption measurements (Acheson *et al.*, 1983, 1988; Astrup *et al.*, 1989; de Fronzo *et al.*, 1984; Welle & Campbell, 1983; Zwillich *et al.*, 1981). In fact a few trials report a reduction, which was probably dependent on the preceding diet (Jung *et al.*, 1980; Welle *et al.*, 1989; Zed & James, 1986). On the other hand, propranolol inhibited significantly the stimulated whole-body respiratory exchange (i.e. 'diet-induced thermogenesis') in numerous trials (Acheson *et al.*, 1983, 1984; Astrup *et al.*, 1989; de Fronzo *et al.*, 1984; Ravussin *et al.*, 1985; Welle *et al.*, 1982, 1983), though there are again contradictory results both in humans (Acheson *et al.*, 1988; Hervey & Tobin, 1983; Morgan *et al.*, 1986; Seaton *et al.*, 1984; Thörne & Wahren, 1989; Vernet *et al.*, 1987; Zwillich *et al.*, 1981) and animals (Dulloo & Miller, 1985).

The exact mode of action by which potassium changes develop in response to medication with a β -adrenoceptor agonist or a β -adrenoceptor antagonist is unclear. It has previously been shown that in exercising human muscle 'acutely' administered terbutaline does not prevent the physiological cellular potassium loss (Rolett *et al.*, 1990) and that propranolol does not increase the release of potassium (Katz *et al.*, 1985). It also seems evident from *in vitro* experiments on rat and mouse skeletal muscle

that β -receptor agonists stimulate the Na-K pump (Clausen & Flatman, 1980; Juel, 1988; Rogus *et al.*, 1977), thereby increasing the transport of potassium into the cell. As to the effect of β -adrenoceptor blockade, Manninen (1970) observed in red cells a bi-directionally accelerated movement mainly of potassium after exposure to very high doses of propranolol *in vitro*, an effect which was not abolished by ouabain.

The experimental *in vitro* conditions for muscle in the present trial were chosen with a physiological external concentration of magnesium, as this has been shown to stimulate glycoside binding to Na-K-ATPase maximally (van Winkle *et al.*, 1972). As in previous 'acute' studies (Gullestad *et al.*, 1991), we found that serum potassium was decreased significantly by continuously administered terbutaline, whereas during treatment with propranolol only a minor increment in serum potassium was seen. Unaltered energy values were recorded in the muscle and no relationship found between changes in extracellular levels of potassium and changes in muscle content of potassium. This result during continuous β -adrenoceptor agonist treatment is not consistent with the hypothesis that decreased serum potassium is due to a chronically activated Na-K pump in resting muscle. It is supported by the finding of Buur *et al.* (1982), demonstrating a markedly suppressed effect *in vitro* of terbutaline or adrenaline on active Na-K transport in soleus muscle from the guineapig after terbutaline pretreatment for 5 days, and indicates a desensitization of β_2 -receptors in muscle.

As in previous studies (Clausen *et al.*, 1991; Fagher *et al.*, 1987) it was found that the lateral vastus muscle under resting conditions utilized only about 6% of its overall energy for the Na-K pump.

In conclusion, direct microcalorimetry of muscle may help to understand the metabolic actions of different β_2 -adrenoceptor agonists. Unexpectedly the present work showed a significant fall in basal heat production from skeletal muscle *ex vivo* after 1 week of oral treatment with sustained release terbutaline in conventional doses. The cause of this metabolic effect is unclear but might be explained by desensitization of β_2 -receptors. Thus in resting muscle an 'energy-saving state', simulating β -receptor blockade, seems to be produced by continuous terbutaline medication. The practical implications of this finding are unknown, especially as clinical situations with higher adrenergic drive are so different from the present basal state. With propranolol a moderate diminution of basal metabolic rate was found at the cellular level, which is in agreement with a number of previous studies using whole-body oxygen consumption measurements. This effect must, however, be distinguished from the clear reduction in 'facultative thermogenesis' that has often been demonstrated under β -adrenoceptor blockade at higher sympathetic tone. Finally, unchanged energy values for the Na-K pump were recorded in the muscle under resting conditions, both during treatment with terbutaline and propranolol.

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