Thermogenesis in human skeletal muscle as measured by direct microcalorimetry and muscle contractile performance during \( \beta \)-adrenoceptor blockade

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Summary

1. The influence of \( \beta \)-adrenoceptor-blockade on skeletal muscle was studied in ten healthy males with propranolol, atenolol and pindolol randomly given for 8 days each in a cross-over double blind test. After 7 days on each drug, muscle function was tested by an isokinetic dynamometer. Thermogenesis in biopsy samples taken from vastus lateralis muscle after a low grade exercise was studied after 8 days on each drug by direct calorimetry with a perfusion microcalorimeter.

2. Before drug administration, a median heat production rate of 0.67 mW/g of muscle was measured. This value was significantly reduced by 25% during propranolol, but no significant change was found during atenolol or pindolol administration.

3. Peak torque decline during isokinetic endurance test changed significantly in knee flexor but not in extensor muscles, from 15% to 27% after propranolol and from 15% to 23% after pindolol. Maximum dynamic strength was unaltered.

4. Our data suggest that blockade of sympathetic \( \beta_2 \)-receptors decreases thermogenesis in human skeletal muscle and impairs isokinetic endurance.

Key words: \( \beta \)-adrenoceptor blockade, heat production, isokinetic contraction, microcalorimetry, skeletal muscle, thermogenesis.

Abbreviations: HR\(_{30} \), heart rate after 30 repeated knee contractions; RPE\(_{30} \), rated perceived exertion.

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Introduction

Evidence indicating a thermogenic effect mediated by \( \beta \)-receptors has arisen from different sources. Temperature regulation in resting subjects exposed to cold conditions was shown to be suppressed by the non-selective \( \beta \)-blocker oxprenolol [1]. The thermogenic response to insulin and glucose infusion, as determined by indirect calorimetry, decreased after intravenously administered propranolol, whereas the basal metabolic rate after an overnight fast was unchanged [2, 3]. In the postprandial state, propranolol has also been shown to reduce the thermogenesis, measured as the inhibitory effect on the increase in metabolic rate that normally occurs after food intake [4]. \( \beta_1 \)-selective blockers have not been studied in a similar way.

Microcalorimetric technique has been usefully applied to different cell systems in the last 15 years (see, e.g., [5, 6]). Recording of heat production by direct calorimetry has not been used, however, in the study of metabolic effects of \( \beta \)-blockers. Our purpose was to evaluate thermogenesis of human skeletal muscle with a microcalorimeter to ascertain whether medication with various \( \beta \)-adrenergic blockers could have an inhibiting influence. Three drugs with widely different pharmacodynamic profiles were chosen: propranolol (non-selective), atenolol (\( \beta_1 \)-selective) and pindolol (non-selective with partial \( \beta_2 \)-agonist activity). Furthermore, muscular strength and dynamic endurance were studied by an isokinetic technique [7]. It also seemed appropriate to see if an altered heat production might possibly be linked to changes in the perception of fatigue, since impaired physical performance and muscle fatigue during \( \beta \)-adrenergic blockade have been a matter of discussion and research in recent years [8–16].
Experimental design

Three drugs were randomly administered for 8 days in a cross-over double blind study: propranolol (80 mg twice daily), atenolol (50 mg twice daily) and pindolol (5 mg twice daily). The three drugs were given as specially prepared identical capsules which were counted for assessment of compliance. Each subject received all three drugs subsequently with at least 1 week intervening between the study periods. Muscle function was tested after 7 days in each period. Muscle biopsies and blood for drug analyses were taken after 8 days on each drug, at the same hour in the morning as the muscle function tests, 2.5 h after drug intake and after a 12 h fast. For comparison, all measurements were also performed before the first medication period. The subjects were encouraged not to change their physical activities and habits during the study. They were informed as to the purpose and possible risks of the investigation and they gave their consent. The study was approved by the Ethics Committee of the University of Lund.

Material and methods

Subjects

Twelve healthy habitually active and non-smoking males were recruited for the study. None of the subjects could be classified as an athlete. Their age was 18–38 years, their height 171–195 cm and their weight 60–85 kg. They took no medication. One subject was withdrawn from the study because of a respiratory tract infection and another subject was excluded owing to non-compliance. The other ten subjects had 100% compliance according to the number of capsules returned.

Tests of contractile performance

Maximum dynamic strength [7, 17] was measured in knee extensor and flexor muscles with an isokinetic dynamometer (Cybex II; Lumex Inc., New York, U.S.A.). The angular velocity during maximum dynamic contraction was controlled at a pre-set level and the velocities studied were 90°/s and 180°/s. Measurements were performed with the subject seated with the hips fixed in 90° flexion. The leg was attached to the lever arm of the dynamometer and the centre of the dynamometer's axis of rotation was aligned with the anatomical axis of rotation of the knee joint. The methodological variation of maximum strength measurements was previously determined to be about 10% [17]. The torque produced by the muscle contraction was calculated and expressed in Nm (force \times the length of the lever arm). The maximum value of three consecutive contractions was used for calculation.

Local muscle fatigue was assessed by 30 repeated maximal isokinetic contractions of the knee extensor and flexor muscles at an angular velocity of 90°/s [18]. Dynamic endurance was calculated as the peak torque decline, expressed as a percentage, from the mean of the three initial contractions and the mean of the three final contractions. Between the contractions there was an approximately 1 s relaxation period. The coefficient of variation was previously reported to be 3.2% at an angular velocity of 180°/s [18]. Rated perceived exertion for the endurance test (RPE30) was studied by asking the volunteers to estimate leg effort on a scale from 6 to 20, according to Borg [19]. Heart rate was measured before and at the end of the endurance test (HR30).

Blood pressure, heart rate and exercise test

On the eighth day on each drug, immediately after drug intake, heart rate and blood pressure were measured after at least 10 min rest in the supine position. The mean of three successive readings was taken. Diastolic blood pressure was indicated by Korotkoff phase V. Mean arterial blood pressure was calculated by adding one-third of the pulse pressure to the diastolic blood pressure. A mercury sphygmomanometer with appropriate cuffs was used. To evaluate the influence of the different drugs, a submaximal exercise test was thereafter performed on an electrically braked bicycle ergometer (Siemens–Elema). The volunteers worked for 4 min on each of the following work loads: 50 W, 100 W, 150 W and 200 W. Heart rate was measured by auscultation with a stethoscope after 4 min on each load. All exercise tests were supervised by the same person (H.L.). Afterwards, the subjects were advised to rest before the muscle biopsy, which was preceded by a new, light exercise.

Muscle biopsy

On four occasions, before and after 8 days on each drug, a muscle biopsy was performed. After local anaesthesia of the skin, the volunteers performed a 4 min exercise with a work load of 50 W. Immediately after the termination of the exercise, while recording the time lag, a biopsy was taken in the supine position from the middle portion of the vastus lateralis muscle by use of the needle technique of Bergström [20]. All biopsies were performed by the same person (B.F.). The muscle
specimens were collected and handled in ice-chilled Krebs—Ringer—phosphate buffer (0.12 mol/l NaCl, 0.005 mol/l KCl, 0.001 mol/l CaCl₂, 0.016 mol/l Na₂HPO₄, 0.001 mol/l MgSO₄, pH 7.40) supplemented with glucose (8.25 mmol/l) and insulin (0.1 unit/ml). Fibre bundles with a diameter of 0.5–1 mm and a resting length of about 4–7 mm were teased away, carefully blotted on filter paper and quickly weighed. Specimens with a weight of about 50 mg were used in the calorimetric experiments (Table 1).

**Calorimetric measurements**

Total metabolic activity in muscle was monitored by measurement of heat production rate by perfusion microcalorimetry with a 0.7 ml flow-through vessel. We have recently described the technique in detail [21]. Measurement was made at 37°C and pH 7.4. A flow of fresh buffer medium (see above) was pumped through the reaction vessel at a rate of 5 ml/h and close to the resting muscle fibre bundles in order to keep pH constant. The thermal power generated by the muscle was calculated from the observed power-time curve and expressed in units of mW/g wet weight. The power values reported are mean values during the second hour after the start of the calorimetric experiments. The coefficient of variation for the method was previously determined to be 4.2%. Time intervals from biopsy to the start of the calorimetric experiments are shown in Table 1.

**Drug analysis**

Venous blood for analysis of β-blockers was sampled both before drug intake and immediately after the muscle biopsy. The blood was collected in heparinized Venoject tubes, centrifuged and stored at −20°C until analysis. Previously described methods were used for determination of propranolol [22], atenolol [23] and pindolol [24].

**Statistical procedures**

Data are presented as medians and interquartile ranges unless otherwise noted. Differences between groups have been tested by Wilcoxon's non-parametric test for paired data. Correlation coefficients were calculated according to Spearman's rank test. *P* values of less than 0.05 were considered as significant.

**Results**

**Muscle heat production**

Before drug administration, the median heat production rate in muscle after the ergometric exercise was 0.67 mW/g with an interquartile range of 0.53–0.75 (Table 1). Neither before nor during medication was any correlation found between heat production and the time lag between the termination of the exercise and the biopsy.

After administration of propranolol, the muscle heat production was 0.46 mW/g (interquartile range 0.28–0.68), which is 25% lower than the premedication level, *P*= 0.02 (Fig. 1). The corresponding values after atenolol and pindolol were 0.62 mW/g (−4%) and 0.53 mW/g (−13%), respectively. These two values were not significantly different from the pre-drug value. Representative calorimetric curves, obtained from one subject before and after medication with propranolol, are shown in Fig. 2.

**Table 1. Premedication characteristics and changes (Δ) after β-adrenoceptor blockade with propranolol, atenolol and pindolol**

Results are median values. Interquartile range is given in parentheses; total range for drug plasma levels.

<table>
<thead>
<tr>
<th></th>
<th>Pre-drug values</th>
<th>Propranolol</th>
<th>Atenolol</th>
<th>Pindolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug plasma levels (nmol/l)†</td>
<td>—</td>
<td>194 (131–530)</td>
<td>1313 (713–1838)</td>
<td>73 (40–133)</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>82 (79–86)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(Δ%)</td>
<td>—</td>
<td>−11 (−11 to 7)**</td>
<td>−13 (−13 to 11)**</td>
<td>−5 (−9 to 3)</td>
</tr>
<tr>
<td>Time of biopsy after exercise (min)</td>
<td>1.3 (1.0–1.3)</td>
<td>1.3 (1.0–1.2)</td>
<td>1.3 (1.1–1.5)</td>
<td>1.3 (1.2–1.3)</td>
</tr>
<tr>
<td>Muscle sample weight (mg)</td>
<td>50.1 (49.8–50.4)</td>
<td>50.1 (49.6–50.5)</td>
<td>49.4 (46.1–49.7)</td>
<td>48.8 (39.7–49.7)</td>
</tr>
<tr>
<td>Start of calorimetric measurement after biopsy (min)</td>
<td>19 (17–22)</td>
<td>20 (20–24)</td>
<td>22 (19–25)</td>
<td>25 (18–30)</td>
</tr>
<tr>
<td>Heat production in muscle (mW/g)</td>
<td>0.67 (0.53–0.75)</td>
<td>0.46 (0.28–0.68)**</td>
<td>0.62 (0.53–0.72)</td>
<td>0.53 (0.40–0.63)</td>
</tr>
<tr>
<td>(Δ%)</td>
<td>—</td>
<td>−25 (−41 to −4)</td>
<td>−4 (−27 to 31)</td>
<td>−13 (−40 to 0)</td>
</tr>
</tbody>
</table>

† At the time of biopsy.
Contractile performance

Results of the muscle function measurements are summarized in Table 2. After \( \beta \)-blockade, maximum dynamic strength of the knee extensor and flexor muscles did not change significantly from the basal premedication value. The median peak torque decline before medication was 29% in knee extensor muscles and 15% in knee flexor muscles. The change in dynamic endurance of the extensor muscles was not significant for any of the drugs, although significant changes were found in the flexor muscles after both propranolol, from 15% to 25% \((P < 0.02)\).

![Fig. 1. Change (%) from initial value in muscle heat production (vastus lateralis) after 8 days of \( \beta \)-adrenoceptor blockade in ten healthy subjects. NS, Not significant. Wilcoxon’s test for paired data.]

![Fig. 2. Representative measurements of thermal power \( (P) \) on biopsy samples from vastus lateralis muscle, obtained from one subject before and after medication with propranolol. Sample ampoule inserted into the microcalorimeter \( i \), and taken out \( \dagger \). \( P_m \) = mean value during the second hour of registration.]

**TABLE 2. Peak torque values of the knee extensor (Ext) and flexor (Flex) muscles before and the change (\( \Delta \)) after \( \beta \)-adrenoceptor blockade in healthy men are shown in (a) dynamic endurance and rated perceived exertion (RPE\(_{30}\)) after 30 knee contractions are given in (b)**

Results are median values. Interquartile range is given in parentheses. Significance versus initial value: *\( P < 0.05 \), **\( P < 0.02 \).

(a)

<table>
<thead>
<tr>
<th>Angular velocity</th>
<th>Maximum strength Pre-drug values (Nm)</th>
<th>Propranolol (( \Delta % ))</th>
<th>Atenolol (( \Delta % ))</th>
<th>Pindolol (( \Delta % ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>90°/s</td>
<td>Ext 163 (127–167)</td>
<td>0 (–9 to 10)</td>
<td>9 (–9 to 19)</td>
<td>1 (–6 to 20)</td>
</tr>
<tr>
<td></td>
<td>Flex 97 (77–108)</td>
<td>9 (–2 to 26)</td>
<td>5 (2 to 27)</td>
<td>8 (–9 to 29)</td>
</tr>
<tr>
<td>180°/s</td>
<td>Ext 111 (92–125)</td>
<td>10 (–2 to 23)</td>
<td>6 (–15 to 16)</td>
<td>10 (–15 to 25)</td>
</tr>
<tr>
<td></td>
<td>Flex 79 (67–83)</td>
<td>15 (13 to 27)</td>
<td>8 (3 to 12)</td>
<td>6 (–10 to 18)</td>
</tr>
</tbody>
</table>

(b)

<table>
<thead>
<tr>
<th>Dynamic endurance (peak torque decline in %) Pre-drug values</th>
<th>Propranolol</th>
<th>Atenolol</th>
<th>Pindolol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flex 15 (13–21)</td>
<td>25 (23–30)**</td>
<td>15 (11–26)</td>
</tr>
<tr>
<td>RPE(_{30})†</td>
<td>(Score) 16 (15–17)</td>
<td>16.5 (15–19)</td>
<td>17 (16–17)</td>
</tr>
<tr>
<td>(( \Delta ))</td>
<td>1 (0–1.8)</td>
<td>1 (0–1.8)</td>
<td>1.5 (0–2.0)*</td>
</tr>
</tbody>
</table>

† At the last of 30 contractions.
Receptor blockade and muscle thermogenesis

Fig. 3. Dynamic endurance (peak torque decline during 30 repeated contractions) tested in knee flexor muscles after $\beta$-adrenoceptor blockade. Results are expressed as change from initial value.

and pindolol, from 15% to 23% ($P < 0.05$) (Fig. 3). The force decline in knee flexor muscles showed no correlation with the decrease in heat production of the vastus lateralis muscle.

Perceived exertion

Six subjects reported increased $\text{REP}_{30}$ values after each medication period. However, the change in $\text{RPE}_{30}$ was statistically significant for pindolol only, with a median increase of 1.5 Borg scale units ($P < 0.05$) (Table 2). Changes in the perception of muscular effort during $\beta$-adrenoceptor blockade did not correlate with changes in dynamic endurance or in muscle heat production.

Heart rate and blood pressure

Resting heart rate decreased significantly during treatment with propranolol and atenolol ($P < 0.01$), and with pindolol in the sitting position only ($P < 0.05$) (Fig. 4, Table 1). Heart rate was also significantly reduced by all three drugs during the ergometric exercise as well as after the endurance test. However, pindolol was less effective than propranolol and atenolol, especially at low grade ergometric work (50–100 W). Resting mean arterial blood pressure is shown in Table 1. Diastolic blood pressure was significantly lowered by all three drugs, whereas the systolic blood pressure was lowered by atenolol only ($P < 0.05$).

Plasma drug concentrations

The drug levels 2.5 h after drug intake are shown in Table 1. There was no correlation between drug plasma levels, sampled immediately before and 2.5 h after drug intake, and heat production in muscle, heart rate or mean arterial blood pressure.

Discussion

Thermogenesis in biopsy samples from vastus lateralis muscle was found to be significantly decreased by non-selective $\beta$-adrenoceptor blockade but not by $\beta_1$-selective blockade. Thus, mainly $\beta_2$-receptors seem to be involved in the process. The lack of thermogenic inhibition after medication with the non-selective $\beta$-blocker pindolol was probably due to the marked partial agonist activity on $\beta_2$-adrenoceptors inherent in this drug. The degree of receptor stimulant effect of pindolol differs from one tissue to another [25]. In human lymphocytes, for example, only pindolol was shown to stimulate cyclic AMP generation, in contrast to three other $\beta$-blockers with pharmacologically different properties [26]. As far as we know, there are no such data for human skeletal muscle. On the other hand, propranolol has been shown to produce a decrease in cyclic AMP content in human skeletal muscle [12].
Our finding, by direct calorimetry, of a reduced metabolic heat production in skeletal muscle during propranolol medication, is in agreement with indirectly obtained data [2–4]. The causal mechanisms for this has received little attention. It seems likely that the adrenergic system is involved with a direct retardation of glycogenolysis. A decreased activity of glycogen phosphorylase \(a\) in human muscle after propranolol was recently shown, both at rest and after short intense work [12]. There is also evidence that the degradation of glycogen is mediated by \(\beta_2\)-receptors (see e.g. [13]), down to lactate, leading to energy production and liberation of heat.

To evaluate the effects of \(\beta\)-blockers, the doses and plasma drug levels have to be taken into consideration. The muscle responses were found to be unrelated to the plasma concentrations of the drugs. Equipotent doses of propranolol and atenolol were administered, as judged from the almost identical effects on heart rate and mean arterial blood pressure. In quantifying the relative \(\beta\)-adrenoceptor blocking potency of pindolol, methodological problems arise when it is compared with drugs devoid of partial agonist activity [27]. At rest and at low and intermediate work loads, less reduction in heart rate is to be expected with pindolol, reflecting the \(\beta_2\)-receptor stimulant effect at low sympathetic tone. At more intense work, when sympathetic tone is higher, the difference in heart rate in response to atenolol and propranolol will consequently be less or minimal, as in the submaximal exercise test in the present study or at the end of the isokinetic endurance test (Fig. 4). It is therefore not surprising that pindolol in circumstances with comparatively less pronounced agonist activity, induced an almost similar change in peak torque decline as propranolol (Fig. 3). This is again evidence for mainly a \(\beta_2\)-receptor mediated influence on skeletal muscle, as \(\beta_2\)-selective blockade had no significant effect on peak torque decline.

\(\beta_2\)-Receptor blockade did not affect maximum voluntary contraction strength whereas, in the isokinetic endurance test, an influence was found on flexor muscles of the knee. The fact that no effect was found on knee extensor muscles might be explained by the fibre-type composition, as the hamstring muscles in man have more glycogenolytic type II fibres than the quadriceps muscle [28].

As judged from the comparison of the perception of discomfort in the exercising muscles (RPE\(_{\text{ex}}\)), analysed and rated according to the Borg scale, and the objective way of measuring fatigue as change in peak torque decline, propranolol gave diverging results. However, as many factors are responsible for the subjective feeling of fatigue [9, 29] and the number of subjects studied was small, the change in clinical scores must be interpreted with great caution.

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**References**


