β-Adrenoceptor blockade and skeletal muscle energy metabolism during endurance exercise

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Van Baak, M. A., A. de Haan, W. H. M. Saris, E. van Kordelaar, H. Kuipers, and G. J. van der Vusse. β-Adrenoceptor blockade and skeletal muscle energy metabolism during endurance exercise. J. Appl. Physiol. 78(1): 307–313, 1995.—Twelve healthy male volunteers cycled to exhaustion at a workload corresponding to 70% of maximal aerobic power after administration of 80 mg of the β1,2-adrenoceptor antagonist propranolol and after administration of placebo by mouth. Exercise times until exhaustion were 38 ± 7 and 86 ± 7 min in the propranolol and placebo groups, respectively. Muscle inosine 5′-monophosphate content was significantly increased above resting levels at exhaustion after placebo. At exhaustion after propranolol, inosine 5′-monophosphate was not increased significantly and was lower than at exhaustion after placebo. No changes in ATP and the total adenine nucleotide content during exercise were found in the two tests. Muscle glycogen content was significantly reduced at exhaustion after placebo as well as after propranolol, but the levels were still significantly higher at exhaustion after propranolol than after placebo. No evidence for a shift in glycogen utilization among types I, IIA, and IIB fibers after propranolol was found. The results show that neither an imbalance between ATP utilization and ATP regeneration nor premature glycogen depletion, either in the whole muscle or in specific muscle fiber types, provides a satisfactory explanation for the premature fatigue during endurance exercise after propranolol.

β-ADRENOCEPTOR blocking agents reduce endurance exercise performance in healthy volunteers as well as patients with hypertension on β-blocker treatment (8, 10, 21, 30, 31, 33). The mechanism of this impairment is incompletely understood. It has been hypothesized that the effects of β-adrenoceptor blocking agents on energy metabolism are involved (29).

Glycogen is an essential energy substrate during prolonged submaximal exercise, and a low muscle glycogen content may limit the rate of glycogenolysis and may therefore result in an imbalance between the utilization and resynthesis of ATP (5). Such an imbalance leads to activation of AMP deaminase and production of inosine 5′-monophosphate (IMP) and its further breakdown products (5, 25). An alternative, or additional, explanation for the IMP accumulation near exhaustion from prolonged exercise could be an increased recruitment or activation intensity of type II muscle fibers (25). It has been shown that an increasing number of type II muscle fibers is recruited during submaximal exercise when the subject is nearly fatigued (11). Moreover, animal studies have shown that type II fibers have a higher maximal AMP deaminase activity than type I fibers and that type II fibers accumulate more IMP than type I fibers under conditions of extreme energetic stress (28).

The β-adrenoceptor in skeletal muscle is of the β2-type (19). One of the effects of β2-adrenergic stimulation of human skeletal muscle at rest is an increased glycogenolysis (12, 26). Adrenergic stimulation of skeletal muscle during exercise by local intra-arterial infusion of a β-agonist increased lactate release from the active muscle (13) and tended to increase muscle glycogen breakdown (16). Therefore, a reduction of muscle glycogenolysis during exercise can be anticipated after β2-adrenoceptor blockade. Indeed, Chasiotis et al. (7) concluded from indirect measurements that the maximal glycogenolytic rate, induced by a supramaximal exercise intensity, decreased after intravenous propranolol administration. However, other studies showed that skeletal muscle glycogen breakdown during prolonged submaximal exercise did not differ between β-blocked and placebo-treated subjects (8–10, 18).

The density of β2-adrenoceptors has been shown to be higher in type I than in type II muscle fibers in experimental animals (23, 27) as well as in humans (23). In addition, epinephrine infusion stimulates glycogenolysis during electrical stimulation in type I but not in type II fibers (12). Therefore, it is possible that after β-adrenergic blockade the rate of glycogen utilization is reduced in type I muscle fibers but shows a compensatory increase in type II fibers either by increased glycogenolysis or by recruitment of more type II fibers.

The present study was undertaken to investigate whether exhaustion from prolonged submaximal endurance exercise after nonselective β-blockade was accompanied by increased breakdown of adenine nucleotides and increased production of IMP to delineate a possible role of an imbalance between ATP formation and utilization in this process. Moreover, the study investigated whether evidence for changes in the glycogen breakdown pattern of different muscle fiber types after nonselective β-blockade could be found.

MATERIALS AND METHODS

Subjects. Twelve healthy male subjects, mean age of 26 (range 21–31) yr, height of 183 (157–200) cm, body mass of 76 (61–93) kg, and maximal oxygen uptake of 54 (41–66) ml·min⁻¹·kg⁻¹ participated in the study. The purpose and risks were explained to the subjects, and all subjects gave written consent before entering the study. The protocol was

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approved by the Ethics Committee of the University of Limburg.

**Experimental design.** Before the actual study, all subjects performed an endurance cycle ergometer test at 70% of their individual maximal aerobic work capacity (W\text{max}) until exhaustion for habituation purposes. In the actual study, the subjects reported to the laboratory twice at 8 A.M. after an overnight fast, with an interval of 1 wk. On arrival, the subjects took their medication (80 mg of the \( \beta \)-blocking agent propranolol before the first test and placebo before the second test) by mouth. The study was single blind. The subjects were unaware of the fixed order of the medications; they were told that they would receive placebo or propranolol in a random order. The design of the study required a fixed order of the medications. After the medication, the subjects were served a light breakfast consisting of two slices of wholemeal bread with butter and cheese or jam, a glass of orange juice, and a cup of coffee or tea.

The exercise tests were performed 90 min after intake of the medication. Before the start of the first exercise test, an incision was made over the vastus lateralis under local anesthesia with lidocaine and a muscle biopsy was taken (rest after propranolol; Rpr). Then the subjects began to exercise on an electromagnetically braked cycle ergometer (Lode) at a work rate corresponding with 70% of the individual W\text{max}. The pedal rate was kept between 80 and 90 rpm. Heart rate (derived from the electrocardiogram) and gas exchange (model 2900, Sensormedics) were averaged over 20 s and were measured continuously during the first 10 min of exercise and for 3 min at the end of every 10-min period thereafter. The average of the last three 20-s periods before a certain time point was taken as the value representing that time point.

During the first test (propranolol) the subjects cycled until exhaustion (EXHpr), which was defined as the moment the subject was no longer able to maintain a pedal rate of >50 rpm. As soon as possible after exhaustion a muscle biopsy was taken from the same incision as the preexercise biopsy with the subject lying down.

The placebo test was performed according to the same protocol. Before the start of the exercise test a resting muscle biopsy was taken again (rest after placebo; Rpl). During the placebo exercise test the subjects stopped exercising at the moment corresponding with the moment of EXHpr (Pt-EXHpr). The subject lay down, and a muscle biopsy was taken. Immediately thereafter (within 3 min), the subject continued to cycle and exercised until exhaustion (EXHpl). Subsequently, a third biopsy was taken. The time delay between end of exercise and freezing of the biopsy varied between 30 and 60 s. The measured nucleotide contents represented the values at the moment the biopsy was frozen.

**Analytic methods.** One portion of the muscle biopsy was quickly frozen in liquid nitrogen for later biochemical analysis; another part was glued to a piece of cork and frozen in isopentane (2-methylbutane) precooled with liquid N\textsubscript{2} for histochemical analysis. The biopsies were stored at −80°C until further analysis.

The biopsies for biochemical analysis were freeze-dried. The wet-to-dry weight ratios were 4.2 ± 0.1 (Rpr), 4.4 ± 0.1 (EXHpr), 4.1 ± 0.1 (Rpl), 4.2 ± 0.2 (Pt-EXHpr), and 4.6 ± 0.1 (EXHpl) and did not differ between placebo and propranolol biopsies. One portion was used for determination of muscle glycogen content; the other was used for determination of muscle nucleotide content.

For biochemical determination of muscle glycogen content, the freeze-dried tissue (8–20 mg) was digested in 1 ml of 1 N NaOH (3 h at 37°C), 1 ml of 95% ethanol was added, and the mixture was heated for 10 min at 85°C. After 20–24 h at 4°C, the samples were centrifuged at 2,500 rpm. Then 1 ml of HCl (1 N) was added to the supernatant, and the mixture was heated for 3 h at 100°C. After cooling, 0.5 ml of tris(hydroxymethyl)aminomethane/KOH (0.12 M/0.1 M saturated with KCl) was added and glucose was determined enzymatically. Glycogen content was expressed as micromoles of glucose per gram dry weight. Glycogen content data at all five time points were available and are reported for 10 subjects.

Tissue nucleotides were determined with a high-performance liquid chromatography technique (34). In short, the freeze-dried tissue was extracted at −15°C in a mixture of perchloric acid (3.0 M) and dithiothreitol (5 mM). After the tissue was ground in the extraction fluid with a glass rod and subsequent centrifugation at 4°C with 1,200 g for 5 min, the supernatant was neutralized with H\textsubscript{2}CO\textsubscript{3}. Aliquots of the neutralized supernatant were injected on a reversed-phase column (Lichrosorb RP-18, Merck) and eluted by gradient elution at a flow rate of 0.8 ml/min. Elution was started with solvent A (an aqueous buffer of NH\textsubscript{4}H\textsubscript{2}PO\textsubscript{4} (150 mM, pH 6.0)) for 4 min. Thereafter, a linear gradient was started. At minute 28 the eluent consisted of 85% of solvent A and 15% of solvent B (a 1:1 mixture by volume of acetonitrile and methanol). Peaks were detected at 254 nm. Complete data were obtained and reported for seven subjects.

For histochemical analysis of the biopsies, serial cross-sections (10 µm thick) were cut in a cryostat at −20°C and mounted on glass slides. For identification of fiber types I, IIa, and IIb, the sections were stained with myofibrillar adenosinetriphosphatase (25 min, pH 9.4) after preincubation in an acid buffer (10 min, pH 4.77) (6, 20). Sections were stained for glycogen with the periodic acid-Schiff (PAS) reaction (35). The staining intensity of the individual fibers was determined by measurements of light transmittance with a microscope equipped with a Zeiss photometer SF. For each fiber the relative optical density of core staining was calculated. Three biopsies did not contain enough fibers to analyze. From the remaining biopsies (n = 57), 150 fibers were analyzed in each section. Types I and IIa fibers were abundant in all sections, whereas no type IIb fibers could be detected in 13 sections. The mean number of type IIb cells in the remaining sections was rather low (17 [range 1–50]). Data of type IIb fibers should therefore be considered with care. For comparison of both methods of glycogen determination (biochemical and histochemical), the biochemically obtained glycogen content for each biopsy was compared with the mean absorption of the 150 fibers in each section. Glycogen content data in types I and IIa muscle fibers at all five time points were available and are reported for nine subjects. For type IIb fibers, the number of subjects with complete data (3) was very small and data on varying numbers of subjects are reported.

**Statistics.** Significant differences were determined with repeated-measures analysis of variance or two-sided Student’s t-tests for paired observations. Post hoc pairwise comparisons were made using Fisher’s protected least-significant difference tests. Statistical significance was set at P < 0.05. Values are reported as means ± SE.

**RESULTS.**

**Endurance and cardiorespiratory data.** Endurance time, i.e., time until exhaustion, was significantly shorter with propranolol than with placebo in all subjects (39 ± 7 vs. 86 ± 6 min, respectively; P < 0.001). The average decrease was 54% (range 17–83%). The reduction in endurance performance showed no significant correlation with the percentage of type I muscle fibers (r² = 0.03).
The administered dose of propranolol caused a high degree of β-adrenoceptor blockade as evident from the reduction of exercise heart rate (minute 10; 117 ± 2 vs. 158 ± 2 beats/min; P < 0.001; Fig. 1A). Minute ventilation was increased after propranolol (Fig. 1B). The increase was statistically significant over the first 10 min of exercise (P < 0.05); at later time points the differences between placebo and propranolol were not statistically significant. The average oxygen uptake over the first 10 min of exercise was significantly reduced after propranolol (P < 0.05), but no statistically significant differences were found at later time points (Fig. 1C). With propranolol, the average respiratory exchange ratio over the first 10 min of exercise was increased (P < 0.001) and remained elevated during the rest of the test although the differences at minute 20 and minute 30 did not reach statistical significance (Fig. 1D).

**Muscle nucleotides.** Muscle IMP content was significantly increased at EXHpl (Rpl vs. EXHpl; P < 0.05; Table 1).

### Table 1. Parameters of muscle energy metabolism

<table>
<thead>
<tr>
<th></th>
<th>Rpr</th>
<th>EXHpr</th>
<th>Rpl</th>
<th>PI-EXHpr</th>
<th>EXHpl</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>26.2±2.4</td>
<td>23.7±1.0</td>
<td>26.1±2.1</td>
<td>24.5±0.9</td>
<td>24.7±1.3</td>
<td>NS</td>
</tr>
<tr>
<td>ADP</td>
<td>2.74±0.05</td>
<td>2.97±0.28</td>
<td>3.15±0.20</td>
<td>2.98±0.16</td>
<td>3.27±0.12</td>
<td>NS</td>
</tr>
<tr>
<td>AMP</td>
<td>0.09±0.01</td>
<td>0.10±0.02</td>
<td>0.14±0.04</td>
<td>0.10±0.01</td>
<td>0.10±0.01</td>
<td>NS</td>
</tr>
<tr>
<td>TAN</td>
<td>26.1±2.4</td>
<td>26.8±1.2</td>
<td>26.4±2.2</td>
<td>27.8±1.0</td>
<td>28.1±1.3</td>
<td>NS</td>
</tr>
<tr>
<td>IMP</td>
<td>0.30±0.08</td>
<td>0.54±0.07</td>
<td>0.41±0.06</td>
<td>0.55±0.12</td>
<td>1.47±0.58*</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Inosine</td>
<td>0.08±0.04</td>
<td>0.32±0.08†</td>
<td>0.07±0.04</td>
<td>0.19±0.04</td>
<td>0.32±0.08†</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>NAD</td>
<td>1.61±0.17</td>
<td>1.70±0.10</td>
<td>1.79±0.18</td>
<td>1.69±0.06</td>
<td>1.90±0.09</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are means ± SE and are given in μmol/g dry wt. TAN, total adenine nucleotide pool; IMP, inosine 5'-monophosphate; Rpr, rest after propranolol; EXHpr, exhaustion after propranolol; Rpl, rest after placebo; PI-EXHpr, time after placebo corresponding with EXHpl; EXHpl, exhaustion after placebo. *Significantly different compared with Rpl, Rpr, PI-EXHpr, and EXHpr, P < 0.06. †Significantly different compared with Rpr and Rpl, P < 0.06 (Fisher’s protected least significant difference test).
FIG. 2. Muscle glycogen (means ± SE) during prolonged submaximal exercise until exhaustion after placebo and propranolol administration (n = 10). R, rest. * Significantly different from R biopsies. † Significantly different from R biopsies and EXH pr biopsies (P < 0.05; Fisher's protected least significant differences test).

Table 1). There were no statistically significant differences in IMP content between Rpl and Rpr or EXHpr and PI-EXHpr. Muscle insine content was significantly increased at EXHpr (Rpr vs. EXHpr; P < 0.05) and EXHpl (Rpl vs. EXHpl; P < 0.05). Differences between EXHpr and PI-EXHpr were not statistically significant. The content of adenine nucleotides (ATP, ADP, and AMP), total adenine nucleotides (TAN), and NAD showed no statistically significant changes over time and did not differ between placebo and propranolol.

Muscle glycogen. Total muscle glycogen content decreased during exercise under both conditions (Fig. 2). There were no statistically significant differences in the resting glycogen level with and without propranolol (346 ± 48 vs. 366 ± 36 μmol/g dry wt, respectively) or between the glycogen level at EXHpr and PI-EXHpr (239 ± 50 vs. 221 ± 44 μmol/g dry wt, respectively). The change in glycogen content over time after propranolol (EXHpr – Rpr) tended to be smaller than that after placebo (PI-EXHpr – Rpl) (106 ± 19 vs. 150 ± 22 μmol/g dry wt, respectively; 0.05 < P < 0.10). Glycogen content was significantly higher at EXHpr than at EXHpl (239 ± 50 vs. 124 ± 31 μmol/g dry wt, respectively; P < 0.01). There were significant linear relationships between exercise time and glycogen utilization after propranolol (r² = 0.61; P < 0.01; n = 12) and after placebo (r² = 0.42; P < 0.01; n = 22) (Fig. 3).

The correlation between the mean muscle glycogen content determined histochemically as optical density of the PAS stain and the glycogen content of the muscle biopsies determined biochemically was high (r² = 0.74; P < 0.001; Fig. 4).

Glycogen content showed a gradual decrease during exercise in types I and IIA muscle fibers after placebo. In type IIb fibers, glycogen breakdown was only statistically significant during the second part of the exercise test (Fig. 5). After propranolol, the decrease in glycogen content in the three fiber types failed to reach statistical significance, which is probably due to the variability inherent to the PAS absorption technique and the small number of subjects. The glycogen content in types I and IIA muscle fibers was significantly lower at EXHpl than at EXHpr (P < 0.05). There were no indications that glycogen utilization was affected differently in types I and II fibers after propranolol.

DISCUSSION

The two main new findings of this study are that 1) EXHpr from submaximal endurance cycle ergometer exercise is not associated with an imbalance between ATP utilization and regeneration and 2) glycogen breakdown in types I, IIA, and IIb muscle fibers during

FIG. 3. Relationship between exercise time and muscle glycogen utilization after placebo (A) and after propranolol (B) administration. Placebo: y = 1.74x + 94.5, r² = 0.42, P < 0.01; propranolol: y = 2.56x + 18.2, r² = 0.61, P < 0.01.
prolonged exhaustive exercise is unaffected by nonselective \( \beta \)-adrenoceptor blockade.

**\( \beta \)-Adrenoceptor blockade, endurance, and cardiorespiratory variables.** The decreased heart rate during exercise confirms the expected high degree of \( \beta \)-blockade after administration of a single dose of 80 mg of propranolol. Propranolol significantly impaired endurance performance (54%) on the upper limit of the range previously found in other studies with nonselective \( \beta \)-adrenoceptor blocking agents (25-51%: Refs. 29, 33). The susceptibility of subjects for the effects of \( \beta \)-blockade on endurance performance varied considerably; the reduction ranged between 17 and 63%. However, the data do not support the suggestion (8, 18) that the reduction in endurance performance of an individual is related to the proportion of type I muscle fibers in the muscles (vastus lateralis).

Oxygen consumption increased more slowly after propranolol than after placebo in the first minutes of exercise. A slowing of the oxygen uptake kinetics by \( \beta \)-adrenoceptor blockade has previously been demonstrated by more sophisticated breath-by-breath analysis (14, 15). In these studies it was also shown that oxygen uptake normalizes after a few minutes, which is in agreement with the results obtained in the present study. A lower oxygen uptake during the first minutes of exercise indicates a more extensive reliance on anaerobic processes for energy production. The increased ventilation in combination with the increased respiratory exchange ratio during this time period supports an increased \( \text{H}^+ \) production, and thus more pronounced anaerobic metabolism leading to lactate formation, after propranolol. Ventilation and respiratory exchange ratio remained slightly elevated during the whole exercise test. An increased respiratory exchange ratio during prolonged submaximal exercise has also been found in other studies (1, 30), although often the increase in respiratory exchange ratio does not reach statistical significance (31, 33, 37) as in the second part of this study. An increased respiratory exchange ratio during endurance exercise after \( \beta \)-blockade suggests a greater reliance on carbohydrate utilization. This could be due to the fact that \( \beta \)-adrenoceptor blockade inhibits adipose tissue lipolysis and possibly also skeletal muscle lipolysis (2, 8, 36). This results in a reduced availability of fatty acids for energy production.

**Muscle nucleotides.** An increase in muscle IMP concentration occurs when the capacity to repolymerize ADP is impaired, which creates an imbalance between the rates of ATP utilization and synthesis, and thus is
indicative of energy shortage in the muscle. In agreement with previous studies (5, 25), an increased muscle IMP content was found at EXHpl from endurance exercise. TAN did not change significantly, which has been shown previously (25, 25). Broberg and Sahlin (5), on the other hand, found a significant reduction of TAN at exhaustion from prolonged exercise. These discrepancies may be due to the fact that it may be difficult to demonstrate small changes in the large TAN pool in the muscle in small populations.

Broberg et al. (4) demonstrated that propranolol increased IMP production and TAN loss during high-intensity exercise (94% of maximal oxygen uptake) of short duration. They suggest that the increased rate of AMP deamination, which indicates a more severe imbalance between ATP utilization and resynthesis, may contribute to the decreased work capacity during β-blockade. However, in the present study IMP content showed no statistically significant increase at EXHpr from prolonged submaximal exercise and was lower than at EXHpl. Thus, there are no indications for an increased AMP deamination at EXHpr from prolonged moderate intensity (70% Wmax) exercise. An imbalance between ATP utilization and regeneration therefore does not seem to contribute to the decreased work capacity in β-blocked subjects during this type of exercise, in contrast to high-intensity exercise of short duration, as suggested by Broberg et al. (4). These data also suggest that the increased plasma NH₃ concentration that is found at EXHpr from endurance exercise (32) is not due to increased deamination of AMP but must come from other sources, e.g., deamination of amino acids, or is due to reduced hepatic and/or renal clearance of NH₃ (3).

Muscle glycogen utilization. The data presented in Fig. 2 show that muscle glycogen content at EXHpr did not differ from the values obtained after the same exercise time on placebo. This suggests that glycogen utilization was similar in both tests, since resting values were not different between placebo and propranolol. Other studies also failed to detect a significant difference in total muscle glycogen breakdown during endurance exercise with or without β-adrenoceptor blockade (8, 10). From the fact that muscle glycogen content was significantly lower at EXHpl than at EXHpr, it can be concluded that EXHpr was not related to low muscle glycogen. Moreover, no evidence was found for a shift in glycogen breakdown between fiber types with propranolol. Biopsies with very low PAS absorption (<5) in certain fiber types were seen in two subjects at EXHpr but in seven subjects at EXHpl. This indicates that fiber-type specific glycogen depletion also did not play a role in the impaired endurance performance after propranolol administration.

In conclusion, the present study shows that muscle IMP content at EXHpr is significantly less than at EXHpl, whereas muscle glycogen content is significantly higher at EXHpr than at EXHpl in total muscle as well as in the different muscle fiber types. Thus, neither an imbalance between ATP utilization and ATP regeneration nor premature glycogen depletion, either in the whole muscle or in specific muscle fiber types, is able to explain the premature fatigue during endurance exercise after propranolol.

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