Metabolic and Hormonal Responses to Adrenoceptor Antagonists in Exercising Rats

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α- and β-adrenoceptors play a key role in the regulation of nutrient supply to working muscles during exercise. To assess their influence in the regulation of substrate utilization, rats were studied during α- or β-adrenoceptor blockade. Energy metabolism was studied by means of indirect calorimetry before, during, and after moderate swimming exercise. Blood samples were taken for the determination of nutrient and hormone concentrations. In addition, central venous blood samples were withdrawn for determination of blood gases, pH, and total hemoglobin concentration (c/Hb). α- and β-adrenoceptor blockade decreased the rates of energy expenditure (EE) and fat oxidation (fat-ox) during and after swimming in comparison to swimming without adrenoceptor blockade. The oxidation of carbohydrates (CHO-ox) was increased in both cases. α-Blockade prevented the exercise-induced increase in blood glucose, plasma free fatty acids (FFA) were not affected, and plasma insulin, norepinephrine (NOR), epinephrine (EPI), and lactate were markedly increased. β-adrenoceptor blockade prevented the exercise-induced increases in blood glucose and FFA. EPI increased slightly more than and NOR less than in the control experiment. The exercise-induced decrease in insulin was more pronounced after β-blockade. α-Blockade caused a less pronounced decrease in venous oxygen saturation (So_2) and tension (Po_2) than in the control experiment. The exercise-induced increase in carbon dioxide tension (Pco_2) was almost absent. After β-blockade, venous So_2 and Po_2 decreased more and Pco_2 increased more than in the control experiment. It is concluded that both α- and β-blockade restrict the rate of EE during exercise. In the case of β-blockade, this may be due to an insufficient supply of nutrients, mainly FFA, although the oxygen supply to the muscles also seems to be diminished by inhibition of the exercise-induced increase in cardiac output and prevention of β-adrenergically mediated vasodilatation in working muscles. In the case of α-blockade, the restriction of energy metabolism seems mainly to be due to an insufficient supply of oxygen caused by prevention of redistribution of cardiac output mainly resulting from insufficient vasoconstriction in the visceral vascular bed.

THE SYMPATHETIC NERVOUS system plays an important part in the supply of energy substrates to muscle tissue during exercise. Epinephrine (EPI) from the adrenal medulla and norepinephrine (NOR) from the nerve endings are key mediators in the regulation of energy substrate supply (for reviews, see Galbo[1] and Scheurink and Steffens[2]). They act on nutrient supply by changing blood flow to the muscles and by changing blood concentrations of glucose and free fatty acids (FFA). Blood flow is modulated by changing cardiac output and by redistribution. Both EPI and NOR cause vasoconstriction in the skin and splanchnic vessels, directing the blood to the working muscles, in which vasodilatation is induced by adrenergic stimulation of β_2-adrenoceptors.[3]

Direct catecholaminergic stimulation of hepatic glycogenolysis in the rat has been reported to be mediated by α-adrenoceptors rather than by β-adrenoceptors.[4,5] Administration of the α-blocker phentolamine in swimming rats prevented the exercise-induced increase in blood glucose, confirming the α-adrenergic stimulation of hepatic glycogenolysis.[6] Administration of the β-blocker timolol in swimming rats also led to a reduction in blood glucose during swimming.[6] However, β-adrenoceptors are likely not involved in the stimulation of hepatic glycogenolysis, because Winder et al[7] did not find an increase in hepatic cyclic adenosine monophosphate in running rats. Therefore, it has been suggested that the reduced blood glucose concentrations observed after β-blockade are due to an enhanced rate of carbohydrate oxidation (CHO-ox) caused by the reduced availability of FFA.[6] Lipolysis in white adipose tissue has been found to be stimulated through β_3-adrenoceptors[8]; this may explain the observed decrease in FFA after β-blockade in swimming rats.[6]

In addition, circulating glucose and FFA concentrations are indirectly influenced by EPI and NOR through activation of α-adrenoceptors on the pancreatic β cell,[9-11] causing an inhibition of insulin release and lower insulin concentrations.[6] This results in decreased rates of hepatic glycogenesis and of lipogenesis in white adipose tissue, leading to higher glucose and FFA concentrations. Another route of indirect action may be via stimulation of β_2-adrenoceptors on muscle tissue. This leads to a higher rate of muscle glycogenolysis[5,12] with consequently diminished uptake of blood glucose; muscle glycogen is the main source of glycolytic substrate in muscle during short-term exercise (< 1 hour).[13,14]

The role of peripheral adrenoceptors in controlling glucose and FFA production is fairly well known. However, the part they play in controlling glucose and FFA utilization is less clear. Therefore, we investigated the utilization of glucose and FFA in swimming rats by measuring oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$), from which energy expenditure (EE) and the rates of CHO-ox and fat-ox were calculated. This was performed after either α- or β-blockade and in the control situation without any blockade.

As mentioned above, some of the effects of α- and β-blockade on metabolism may be attributed to alterations in cardiovascular control. The exercise-induced increase in

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cardiac output is to a large extent due to β-adrenergic chronotropic and inotropic effects on the heart.[3] In addition, exercise-induced vasodilation in muscles is partially under β-adrenergic control,[9,14] and compensatory vasoconstriction in the splanchnic vessels is under α-adrenergic control.[3] Failure of the occurrence either of an increase in cardiac output or of the shift in blood flow from the splanchnic bed to the working muscles will affect oxygen and substrate flow to the working muscles. Therefore, we also measured central venous oxygen tension (P_{O_2}), oxygen saturation (S_{O_2}), carbon dioxide tension (P_{CO_2}), pH, and total hemoglobin concentration (ctHb) before, during, and after exercise in rats with either α- or β-blockade or without interfering with adrenoceptors. Plasma lactate levels were determined to obtain an impression of the contribution of anaerobic glycolysis to EE. Plasma NOR and EPI concentrations were determined during exercise under α- and β-blockade, because previous investigation has shown that stimulation of presynaptic α_2- and β_2-adrenoceptors in sympathetic nerve endings greatly affects NOR release from these nerve endings.[15] Possible changes in plasma NOR and EPI induced by α- and β-blockade thus might contribute to changes in the supply and degradation of energy substrates.

In summary, the aim of this study is to investigate the role of α- and β-blockade on energy metabolism during exercise. Special attention is paid to EE and the contribution of CHO-ox and fat-ox. However, α- and β-adrenergic blockade does not only affect substrate utilization, but also cardiovascular regulation. Therefore, we also estimated shifts in blood flow by measurements of central venous pH, P_{CO_2}, P_{O_2}, and S_{O_2}.

MATERIALS AND METHODS

Animal Care and Surgery

Male Wistar rats weighing 375 to 425 g before the experiments were used. The animals were housed and handled as described previously.[16] They were maintained on a 12-hour light-dark cycle (7 AM to 7 PM light). Surgery was performed under ether anesthesia. All animals were provided with an indwelling catheter in the right jugular vein, with the tip at the entrance of the right atrium and the other end externalized on top of the skull.[17] This method allows frequent sampling of well-mixed central venous blood in unanesthetized, undisturbed, freely moving rats.[18] The experiments started when the animals had regained their preoperative body weight, but at least 1 week of recovery was allowed. To avoid novelty stress, rats were habituated to the experimental conditions in four to five training sessions in which the duration of swimming exercise was gradually increased.[19,20]

Exercise and Gas Analysis

The experiments were performed in an airtight Plexiglas (Vinkkunstoffen BV, Diclam, The Netherlands) swimming pool provided with a metabolic chamber at its upper side. The bottom of the chamber was a movable resting platform that could be lowered into the pool so that the rat was forced to swim to keep its head in the metabolic chamber.[16] A water current of 0.22 m/s was used. The water temperature was 32° ± 2°C. The air flow through the metabolic chamber was 4.5 L/min. $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were measured by means of an O_2 analyzer (Ametek S3A, Pittsburgh, PA) and a mass spectrometer (Balzers QMG 511), respectively, in combination with a gas flow meter. The measuring system was calibrated before each experiment as described previously.[21] In studies using indirect calorimetry, the degradation of proteins can be calculated from nitrogen excreted with urine. In short-term experiments and especially during swimming, collection of urine is hardly possible. Therefore, EE, CHO-ox, and fat-ox were calculated according to the method of Lusk,[22] without taking proteins into account. Although this procedure introduces an error in the absolute rates of EE, CHO-ox, and fat-ox, it has proven its validity for the determination of drug-induced changes in these rates.[16]

Blood Sampling and Chemical Determinations

During an experiment, a series of 0.7-mL blood samples were withdrawn for determination of glucose and lactate concentrations in whole blood, and FFA, insulin, EPI, and NOR concentrations in plasma. For blood sampling, a polyethylene tube (length 400 mm, OD 1.25 mm, ID 0.75 mm) was connected to the outlet of the catheter, pierced through a passage in the hood of the metabolic chamber, and sealed airtight with modeling clay. After a sample was taken, the rat received a transfusion of 0.7 mL citrated (0.6%) donor blood obtained from undisturbed cannulated rats. Between the withdrawal of successive blood samples, the tip of the catheter was filled with 6% citrate solution as an anticoagulant; citrate was used instead of heparin to avoid activation of endothelial lipase.

After withdrawal, blood samples were transferred to chilled (0°C) centrifuge tubes containing 11 μL EDTA solution (70 g/L) as anticoagulant and antioxidant. Blood glucose was determined by an enzymatic photometric method (Sigma, St Louis, MO) using 75 μL blood. The lactate level was measured by an automated enzymatic method in 20 μL blood (Kontron 640, Milan, Italy). The remaining blood was centrifuged for 12 minutes at 2,600× *g* and 4°C. Plasma (100 μL) was used for determination of catecholamines and stored at −80°C until treatment. Catecholamines were determined by high-performance liquid chromatography with electrochemical detection.[15,23] The remaining plasma was stored at −30°C until further handling. FFA levels were measured in 25 μL plasma by an enzymatic photometric method (WAKO, Osaka, Japan). Rat-specific plasma immunoreactive insulin was determined by radioimmunoassay (NOVO, Copenhagen, Denmark). Guinea pig serum M8309 served as antiserum. Duplicate assays were performed on 25-μL plasma samples. Bound and free ^{125}I-labeled insulin was separated by means of a polyethylene glycol solution.[24]

In separate experiments, central venous blood samples (0.5 mL) were obtained for determination of P_{O_2}, P_{CO_2}, pH, S_{O_2}, and ctHb. Also in these experiments, each sample was immediately replaced by 0.5 mL donor blood (0.6% citrate). Immediately after withdrawal in gas-tight heparinized (5 μL, 500 U/mL) syringes, the samples were introduced into an ABL2 blood gas analyzer (Radiometer, Copenhagen, Denmark) for determination of P_{O_2}, P_{CO_2}, and pH, and into a multiwavelength hemoglobin photometer (OSM3, Radiometer) for determination of S_{O_2}, and ctHb.

Adrenoceptor Antagonists

Phentolamine (Regitine; Ciba Geigy, Basel, Switzerland) in a total dose of 0.9 mg, and timolol (Merck, Sharp & Dohme, Rahway, NY) in a total dose of 0.7 mg were used as α- and β-blocking agents, respectively. These doses result in effective blockade of peripheral α- and β-adrenoceptors.[6] The blocking agents were administered together with donor blood after blood sampling at the times and in the amounts shown in Fig 1. The animals received the α-blocker, the β-blocker, or the solvent saline (9 g/L) in random order with at least 1 week between consecutive experiments.

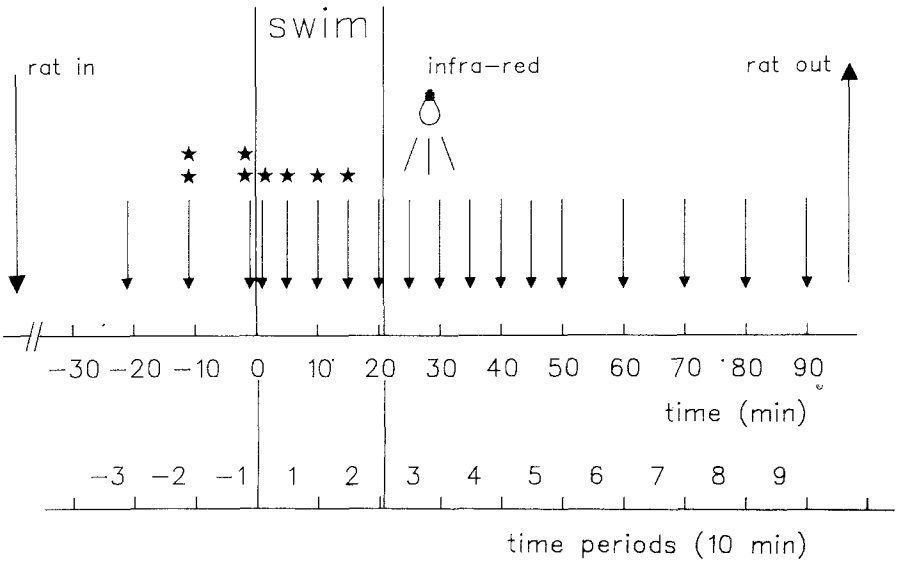

Fig 1. Experimental protocol. Arrows denote the withdrawal of blood samples. *The donation of 12.5% of the total dose of the blocking agent.

Experimental Protocol

All experiments were performed between noon and 4 PM, ie, in the light period. Food was removed about 3 hours before the start of the experiment, and the animal was transferred to the experimental room. After calibration of the measuring system, the animal was placed into the metabolic chamber on top of the swimming pool and connected to the polyethylene tube for blood sampling. The experiment was started when both $\dot{V}O_2$ and $\dot{V}CO_2$ had stabilized, which took approximately 1.5 hours. Blood samples for determination of hormone and energy substrate concentrations and gas tensions were taken at the times shown in Fig 1. At t = 0, the platform was lowered to the bottom of the swimming pool, forcing the rat to swim. At t = 20 minutes, the platform was raised to enable the rat to leave the water. An infrared lamp was switched on to prevent cold stress.

Statistics

Data are expressed as the mean changes ± SE over 10-minute periods, taking the values of period t = 20 to 11 minutes before swimming as baseline. Wilcoxon's matched-pairs signed-rank test was used for comparison of a value at any moment during the experiment with the baseline value. ANOVA and the Mann-Whitney U test were applied to determine any differences between data obtained in experiments with adrenoceptor blockade and data obtained in control experiments. The level of significance was set at P less than .05.

RESULTS

Table 1 presents baseline values for $\dot{V}O_2$, $\dot{V}CO_2$, respiratory quotient (RQ), EE, CHO-ox, and fat-ox as determined in the period t = 20 to 11 minutes before swimming in all experiments. Table 2 presents baseline values for blood glucose, plasma FFA, lactate, insulin, EPI, and NOR in the experiments on energy substrates and hormones. Table 3 presents baseline values for P_{O_2}, S_{O_2}, ctHb, P_{CO_2}, base excess (BE), and pH as determined in the period t = 20 to 11 minutes before swimming in the experiments on blood gases. There are no significant differences between groups. In Fig 2 are presented the mean changes (±SE) from the baseline for $\dot{V}O_2$, $\dot{V}CO_2$, and RQ in control experiments (n = 17) and in experiments with peripheral α- (n = 17) or β-adrenoceptor (n = 17) blockade. In Fig 3 are presented the mean changes (±SE) from the baseline for EE, CHO-ox, and fat-ox; in Fig 4, the mean changes in glucose, FFA, and lactate; in Fig 5, the mean changes in EPI, NOR, and insulin in control experiments (n = 9) and in experiments with peripheral α- (n = 9) or β-adrenoceptor (n = 9) blockade; in Fig 6, the mean changes in P_{O_2}, S_{O_2}, and ctHb; and in Fig 7, the mean changes in P_{CO_2}, BE, and pH in control experiments (n = 8) and in experiments with peripheral α- (n = 8) or β-adrenoceptor (n = 8) blockade.

After α- and β-adrenoceptor blockade, a significant reduction in the exercise-induced increases in $\dot{V}O_2$ and $\dot{V}CO_2$ was observed (Fig 2). After swimming, both quantities remained (in comparison to the control experiment) reduced for two periods. Thereafter, they were at the same level as in the control experiment, except for $\dot{V}O_2$ in periods 5 and 6 in the case of α-blockade and for $\dot{V}O_2$ in period 5 in the case of β-blockade. In the experiment with α-blockade, the RQ was significantly higher than in the control experiment from period −1 through period 9. In the experiment with β-blockade, the RQ was significantly higher than in the control experiment from period 2 through period 8.

α- and β-blockade caused significantly reduced increases in EE during swimming and during the first 30 minutes thereafter in comparison to the control experiment (Fig 3). CHO-ox was significantly augmented in periods 2, 7, 8, and

Table 1. Baseline Values of Quantities Obtained by Indirect Calorimetry

	Control (n = 17)	α-Blockade (n = 17)	β-Blockade (n = 17)
$\dot{V}O_2$ (mmol/kg · min)	0.70 ± 0.02	0.69 ± .02	0.70 ± .02
$\dot{V}CO_2$ (mmol/kg · min)	0.58 ± 0.02	0.55 ± 0.01	0.58 ± 0.02
RQ	0.84 ± 0.01	0.81 ± 0.01	0.83 ± 0.01
EE (W/kg)	5.27 ± 0.13	5.17 ± 0.10	5.26 ± 0.16
CHO-ox (mg/kg · min)	8.5 ± 0.8	6.3 ± 0.7	7.8 ± 0.5
Fat-ox (mg/kg · min)	4.4 ± 0.3	5.2 ± 0.1	4.7 ± 0.3

NOTE. Values are averages ± SE measured in period 2, ie, 20 to 11 minutes before swimming.

Table 2. Baseline Values of Blood Glucose, Plasma FFAs, Blood Lactate, Plasma Insulin, and Plasma Catecholamines

	Control (n = 9)	α-Blockade (n = 9)	β-Blockade (n = 9)
Glucose (mmol/L)	6.08 ± 0.14	6.38 ± 0.29	6.27 ± 0.24
FFA (mmol/L)	0.32 ± 0.04	0.35 ± 0.03	0.37 ± 0.02
Lactate (mmol/L)	1.2 ± 0.1	1.1 ± 0.1	1.2 ± 0.1
Insulin (mU/L)	47 ± 7	53 ± 7	59 ± 8
EPI (ng/L)	22 ± 3	25 ± 2	57 ± 15
NOR (ng/L)	136 ± 37	69 ± 11	99 ± 11

NOTE. Values are averages ± SE obtained at $t = -11$ minutes, ie, before swimming.

9 of the experiment with α-blockade and in periods 1, 2, 7, and 8 in the experiment with β-blockade, as compared with the control experiment. Compared with the control experiment, reduced rates of fat-ox were found in experiments with α-blockade and in those with β-blockade; the reductions were significant from period 1 throughout the experiment in the case of α-blockade and from period 2 throughout the experiment in the case of β-blockade.

After α-blockade, swimming induced a significantly smaller increase in glucose concentration than it did in the control experiment; blood glucose remained reduced during the entire recovery period (Fig 4). In comparison to the control experiment, β-blockade caused significantly reduced glucose levels during swimming and the first two periods thereafter. During the last four periods of the experiment with α-blockade, plasma FFA was at baseline level, a significant reduction as compared with the control experiment. Compared with the baseline value, β-blockade caused significantly decreased FFA levels during swimming. During the first five periods of recovery, plasma FFA was at the baseline level. Then FFA increased to the level observed in the control experiment. Blood lactate appeared to be significantly more increased after α-blockade than in the control experiment; β-blockade did not lead to significant changes.

Insulin was significantly increased (in comparison to the baseline level and the control experiment) from the first injection of the α-blocker until the end of the experiment (Fig 5). Compared with the control experiment, β-blockade caused significantly reduced insulin levels from the second period during swimming until the end of the experiment. EPI increased significantly more during swimming in the experiments with α-blockade than in the control experiments, and remained significantly higher throughout the

Table 3. Baseline Values of Venous Blood Gases, ctHb, pH, and BE

	Control (n = 8)	α-Blockade (n = 8)	β-Blockade (n = 8)
P_{O_2} (kPa)	5.85 ± 0.10	5.84 ± 0.18	5.56 ± 0.19
S_{O_2} (%)	70.0 ± 1.0	67.8 ± 2.0	68.0 ± 2.4
ctHB (g/L)	13.8 ± 0.3	13.4 ± 0.3	13.6 ± 0.4
P_{CO_2} (kPa)	5.87 ± 0.09	6.04 ± 0.11	5.83 ± 0.14
pH	7.43 ± 0.01	7.45 ± 0.01	7.46 ± 0.02
BE (mmol/L)	−1.4 ± 0.9	0.9 ± 1.8	0.6 ± 1.4

NOTE. Data are averages ± SE obtained at $t = -11$ minutes, ie, before swimming.

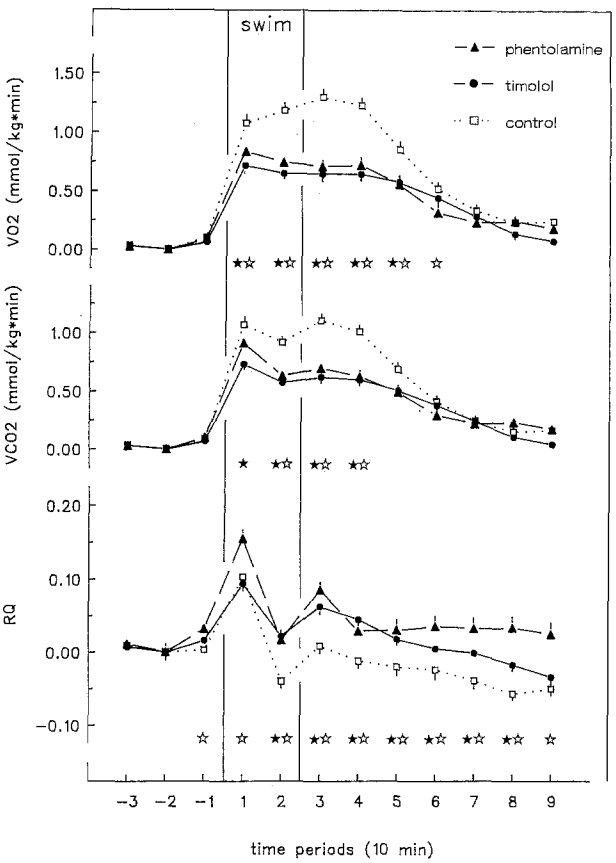

Fig 2. Effect of intravenous administration of the α-adrenoceptor antagonist phentolamine (0.9 mg) and the β-adrenoceptor antagonist timolol (0.7 mg) on $\dot{V}O_2$, $\dot{V}CO_2$, and RQ, in comparison to the control experiment. Data are expressed as average changes (±SE) from baseline levels over 10 minute periods. Baseline levels were measured over period −2, ie, 20 to 11 minutes before swimming and before any drug was administered. Open and closed stars denote significant differences between the experiment with α-blockade and the control experiment and between the experiment with β-blockade and the control experiment, respectively ($P < .05$).

experiment. β-Blockade led to a significantly higher increase in EPI in the second period of swimming. The exercise-induced increase in NOR was much higher after α-blockade than it was in the control experiment; the increase was significantly higher from the first injection of the α-blocker until the end of the experiment. β-Blockade, in contrast, caused a significantly reduced increase in NOR during swimming and the first period thereafter. In periods 5 and 6 of the experiment with β-blockade, NOR appeared to be more increased than in the control experiment.

The decline in S_{O_2} during swimming and recovery appeared to be significantly more pronounced after β-blockade than in the control experiment in periods 1 through 7 (Fig 6). α-Blockade, in contrast, led to a significantly reduced decline in S_{O_2} in periods 1 through 7. The greater decrease in S_{O_2} during β-blockade was reflected in a more pronounced decline in P_{O_2}, which reached the level of significance in periods 1 through 8. In the experiment with α-blockade, both P_{O_2} and S_{O_2} were significantly less reduced in periods 1 through 7. ct Hb was increased during

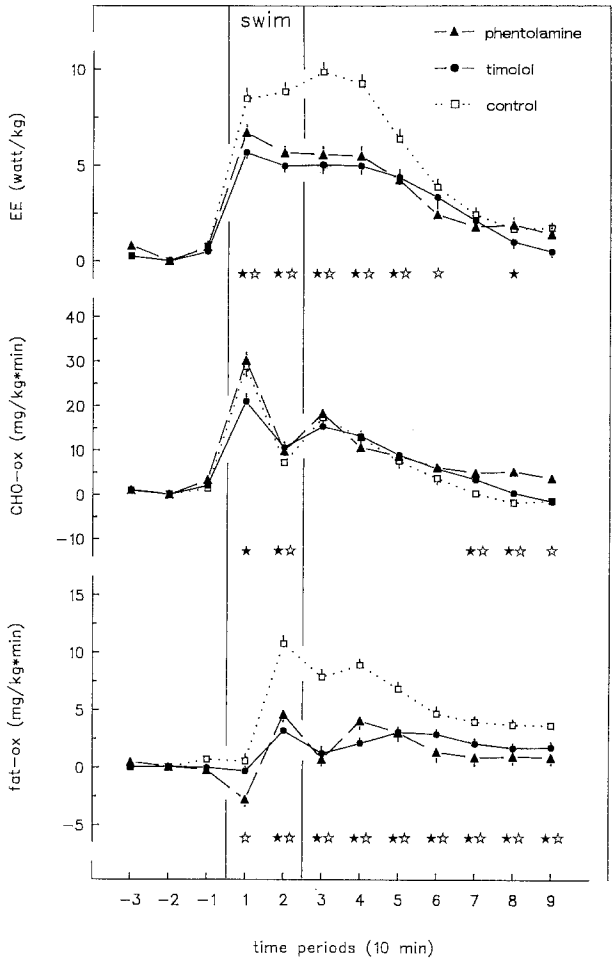

Fig 3. Effect of intravenous administration of the α-adrenoceptor antagonist phentolamine (0.9 mg) and the β-adrenoceptor antagonist timolol (0.7 mg) on EE, CHO-ox, and fat-ox, in comparison to the control experiment. Data are expressed as in Fig 2.

swimming in the control experiment (Fig 6). During recovery, it declined to 10 g/L under the baseline in period 9. β-Blockade led to a significantly higher ctHb level in periods −1, 2 through 6, and 8. α-Blockade caused a significantly lower ctHb level in periods 1, 2, and 3 as compared with the control experiment.

The behavior of P_{CO_2} approached a mirror image of that of P_{O_2}. β-Blockade led to a significantly more pronounced increase in periods 1 through 7. α-Blockade led to a reduced increase in P_{CO_2} from period 1 through period 4. During swimming and the first period of recovery, a decline in pH was observed in the control experiment. In the second period of recovery, pH was at the baseline, and then it increased to slightly above the baseline and remained so throughout the experiment. This is obviously caused by a somewhat increased ventilation, which resulted in a decreased P_{CO_2}. In comparison to the control experiment, β-blockade resulted in significantly decreased pH values in periods 4 through 7. No significant differences in pH with the control experiment were observed in the experiment with α-blockade. In comparison to the control experiment, the experiment with α-blockade showed a significantly more decreased level of BE in periods 1 through 5. β-Blockade did not induce significant alterations in BE as compared with the control experiment (Fig 7).

DISCUSSION

The major findings of our study are as follows: (1) reduced $\dot{V}O_2$, $\dot{V}CO_2$, and EE during and after exercise during α-blockade and β-blockade; (2) decreased fat-ox and increased CHO-ox at most time points during and after exercise during α-blockade and β-blockade; (3) reduced blood glucose levels during and after swimming during α-blockade and temporarily reduced blood glucose levels during β-blockade; (4) complete suppression of an increase in plasma FFA during and after swimming during β-blockade; (5) an exaggerated increase in plasma lactate levels during and after swimming during α-blockade; (6) highly exaggerated increases in both plasma EPI and NOR concentrations during and after swimming during α-blockade, and suppressed plasma NOR levels during β-blockade; (7) exaggerated and suppressed plasma insulin concentrations during and after swimming during α- and β-blockade,

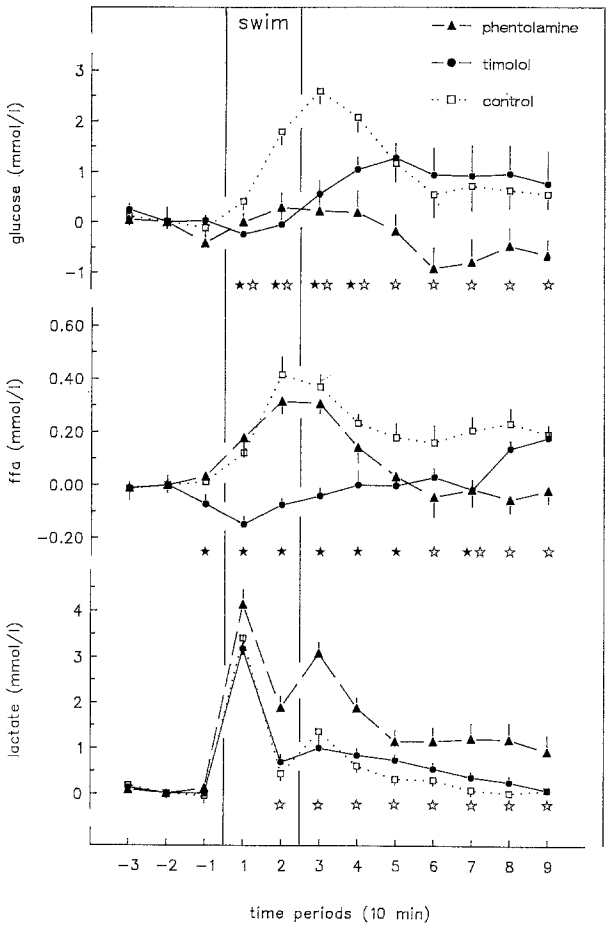

Fig 4. Effect of intravenous administration of the α-adrenoceptor antagonist phentolamine (0.9 mg) and the β-adrenoceptor antagonist timolol (0.7 mg) on blood glucose, plasma FFA, and plasma lactate, in comparison to the control experiment. Data are expressed as in Fig 2.

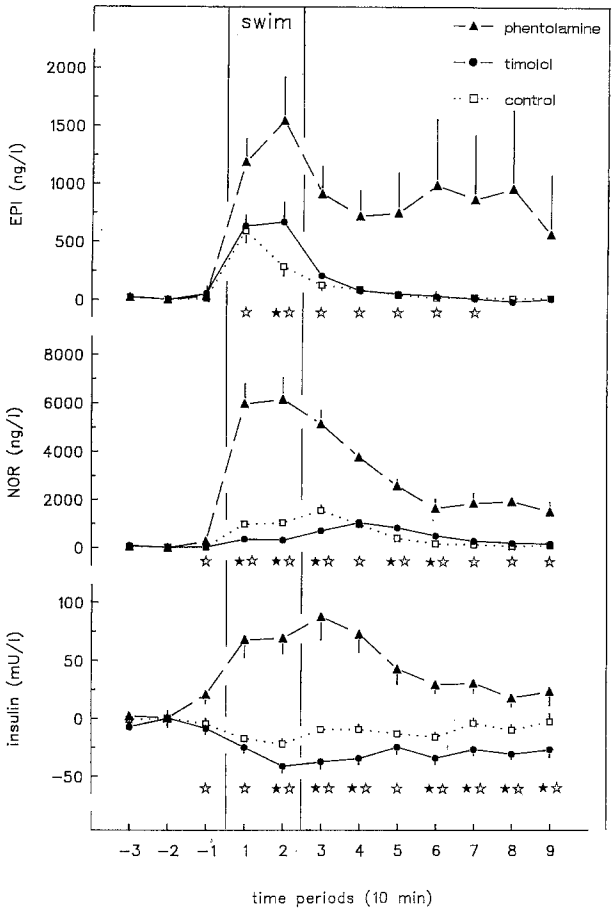

Fig 5. Effect of intravenous administration of the α-adrenoceptor antagonist phentolamine (0.9 mg) and the β-adrenoceptor antagonist timolol (0.7 mg) on EPI, NOR, and insulin in plasma, in comparison to the control experiment. Data are expressed as in Fig 2.

respectively; (8) a reduced decrease in central venous P_{O_2} and S_{O_2} during swimming under α-blockade, accompanied by a significantly increased decrease in BE and a diminished increase in P_{CO_2}; and (9) an exaggerated decrease in central venous P_{O_2} and S_{O_2} during swimming under β-blockade, accompanied by an exaggerated increase in P_{CO_2}.

α-Blockade During Exercise

α-Blockade prevents the exercise-induced increase in blood glucose. This will be first due to blockade of liver glycogenolysis and second to the high plasma insulin levels. Ample evidence is available that in rat liver glycogenolysis is mainly α_1-adrenoceptor-mediated.[4,7,25] The high insulin level during α-blockade is caused by elimination of the α_2-adrenoceptor-mediated suppression of insulin release.[26-28] This α_2-adrenoceptor-mediated suppression is elicited by the increased sympathetic tone during exercise.[26,27] High insulin levels contribute to the decrease in blood glucose, because of increased transport of glucose into insulin-dependent tissues. α-Blockade does not affect lipolysis during exercise, because lipolysis in white adipose cells is β_3-adrenoceptor-mediated.[8,29] Lipolysis may be counteracted by the high insulin levels, but the increased plasma NOR and EPI concentrations occurring during exercise under α-blockade possibly neutralize the insulin effect, as indicated by the nearly normal plasma FFA levels during and immediately after swimming. Because FFA enter the muscle cells by diffusion, one would not expect a change in fat-ox. However, fat-ox is markedly suppressed during and after exercise. Although CHO-ox is somewhat enhanced in this situation, the increase in CHO-ox cannot match the decrease in fat-ox, which accounts for the decreased EE during and after swimming during α-blockade.

The diminished exercise-induced increase in EE under α-blockade thus does not seem to be due to insufficient energy substrate availability. The FFA level is hardly different from that in the control experiment (Fig 4) and α-blockade does not interfere with muscle glycogenolysis, because this is a β_2-adrenoceptor-mediated effect,[5,12,30-32] which can be greatly enhanced due to the high plasma EPI levels since EPI has high affinity for the β_2-adrenoceptor. Therefore, it should be considered whether a diminished oxygen supply might be the underlying factor. The central venous blood gas values suggest that this indeed may be the case. During exercise, P_{O_2} and S_{O_2} remain quite high, while there is only a slight increase in P_{CO_2}, indicating that a

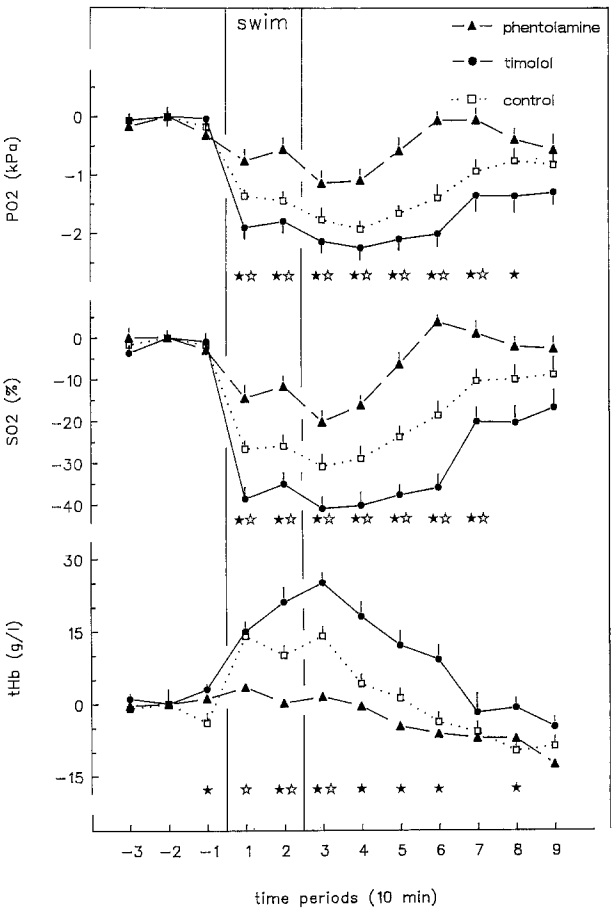

Fig 6. Effect of intravenous administration of the α-adrenoceptor antagonist phentolamine (0.9 mg) and the β-adrenoceptor antagonist timolol (0.7 mg) on central venous P_{O_2}, S_{O_2}, and ctHB, in comparison to the control experiment. Data are expressed as in Fig 2.

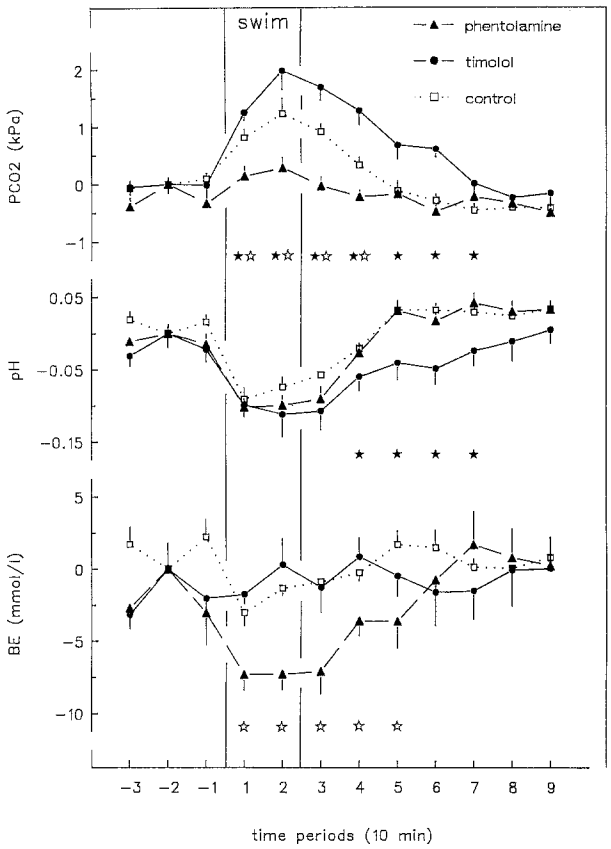

Fig 7. Effect of intravenous administration of the α-adrenoceptor antagonist phentolamine (0.9 mg) and the β-adrenoceptor antagonist timolol (0.7 mg) on central venous P_{CO_2}, pH, and BE, in comparison to the control experiment. Data are expressed as in Fig 2.

major part of the cardiac output passes through relatively inactive tissues. This may be due to suppression of the splanchnic vasoconstriction, normally occurring during exercise,[3] caused by the α-blockade. Consequently, muscle blood flow is compromised. This is corroborated by the increase in blood lactate concentration and the concomitant decrease in BE.

The observed exaggerated plasma EPI and NOR concentrations need explication. In presynaptic membranes of sympathetic nerve endings, α_2- and β_2-adrenoceptors are present.[15,33] Sympathetic activation as occurs during exercise leads to increased release of NOR from the sympathetic nerve endings. This release leads to inhibition of further release of NOR because of stimulation of the inhibiting α_2-adrenoceptor at the sympathetic nerve ending. α-Blockade will eliminate this inhibition, causing NOR release. In addition, NOR release will be stimulated by activation of the β_2-adrenoceptor at the sympathetic nerve ending that elicits NOR release. The high EPI level contributes to this process. Several mechanisms might contribute to the exaggerated plasma EPI levels: relative hypoglycemia, cardiovascular reflexes, and central nervous system mechanisms. Hypoglycemia is a powerful stimulus for the release of EPI from the adrenal medulla,[27] but in this case this cannot be an important factor because β-blockade leads to approximately the same blood glucose concentration as α-blockade, whereas β-blockade does not lead to an exaggerated plasma EPI level (Figs 4 and 5). Alternatively, impeded glycogenolysis in the liver by α-blockade might lead to reflexively enhanced EPI release. The absence of vasoconstriction in the splanchnic bed during swimming under α-blockade as suggested by the high central venous P_{O_2} and S_{O_2} might lead to a decrease in arterial blood pressure. To restore normal blood pressure, sympathetic activity will be reflexively enhanced with consequently increased plasma EPI and NOR levels. Also, central nervous system mechanisms cannot be ruled out, since phentolamine may pass the blood-brain barrier. However, infusion of phentolamine into the ventromedial hypothalamus caused a decreased exercise-induced increase in EPI.[34]

β-Blockade During Exercise

β-Blockade leads to a complete absence of an exercise-induced increase in plasma FFA. Blood glucose levels are temporarily suppressed during and immediately after swimming, after which control levels are soon reached. EE and fat-ox are suppressed during and after swimming, whereas CHO-ox is increased during swimming. However, this increase does not compensate for the suppression of fat-ox. The decreased rate of EE during swimming therefore will primarily be due to the decrease in energy substrate availability. Since FFA enter the muscle cells by diffusion, the availability of FFA as energy substrate is reduced due to the low plasma concentration after β-blockade. The availability of CHO is also decreased; muscle glycogen, the major source of CHO during short-term exercise,[13,14] is available to a limited extent. Although muscle glycogenolysis is stimulated by muscle contraction, it has been found to be stimulated via β_2-adrenoceptors[5,12,30-32] as well. In the present study, the exercise-induced increase in blood glucose concentration was prevented after β-blockade, which cannot be due to a direct inhibition of hepatic glycogenolysis since this process is α_1-adrenoceptor–mediated in the rat.[4,25] That liver glycogenolysis is not prevented by β-blockade is corroborated by the recent findings of Leloux,[35] who found an increase in blood glucose in rats placed in shallow water after β-blockade, demonstrating that blood glucose can be increased after β-blockade. Thus, prevention of the exercise-induced increase in blood glucose in our rats will be the result of an increased utilization of blood glucose, rather than of decreased rates of hepatic glycogenolysis or gluconeogenesis. The latter may anyway be excluded, since the blood lactate concentration was not affected by β-adrenergic blockade, with lactate being a major substrate for gluconeogenesis. Both decreased availability of CHO in the contracting muscle, because of a lack of β_2-adrenoceptor–mediated stimulation of muscle glycogenolysis, and suppression of fat-ox[36] might contribute to enhanced glucose uptake from the circulation. In this connection, it should be noted that contracting muscle cells can take up glucose independently of insulin.[37,38] Plasma insulin is even more suppressed in exercising rats during β-adrenoceptor blockade than in the control situation (Fig 5). This is a confirma-

tion of earlier observations that insulin release from β cells of the islets of Langerhans can be stimulated through β_2-adrenoceptors.[20] The less-pronounced increase in plasma NOR in exercising rats during β-blockade as compared with the control situation can be attributed to a suppression of β_2-adrenoceptor–stimulated NOR release from the sympathetic nerve endings.[15]

Although the diminished availability of FFA seems to be a major factor in the decreased EE in exercising rats during β-blockade, a change in cardiovascular control might contribute to the diminished EE. β-Blockade reduces the increase in cardiac output.[39,40] Besides, β-adrenergically mediated dilatation of the muscular vasculature will be reduced.[9] As a consequence, perfusion of muscle tissue will be decreased, leading to a decrease in oxygen supply. This is reflected in the central venous S_{O_2}, P_{O_2}, and P_{CO_2}. Despite the reduction in $\dot{V}_{O_2}$, both S_{O_2} and P_{O_2} are more reduced during swimming and the first part of recovery than in the control experiment, indicating increased oxygen extraction. Since there is no reason to assume that β-blockade diminishes arterial S_{O_2}[9,41,42] and since ctHb shows an increased exercise-induced increase, the arterial oxygen concentration may even have been increased after β-blockade. Venous P_{CO_2} is more increased during swimming after β-blockade, indicating an increased uptake of CO_2 per unit volume of blood. Also, this is an indication of a diminished blood flow through the working muscles and consequently of a limited oxygen supply, which may have contributed to the limited increase in EE. However, the reduced availability of nutrients seems to be the principal cause, since lactate is not increased after β-blockade and BE did not decrease. The absence of an increase in lactate indicates that the rate of anaerobic glycolysis was not significantly increased.

In conclusion, it can be stated that both α- and β-adrenoceptors play an important part in the control of EE. Blockade of any of the receptor types causes a significant decrease in EE, not only through influencing energy substrate availability but also through affecting oxygen supply, as measurements of central pH and blood gases suggest. With α-blockade, the limited oxygen supply appears to be the principal cause of diminished EE; in this case, the animals have to resort to increased anaerobic glycolysis, as indicated by the increased lactate concentration. With β-blockade, failing release of FFA and elimination of muscle glycogenolysis during swimming will be the main causes of the decrease in EE. This means that the autonomic nervous system, especially the sympathetic division, is involved in the adaptations during exercise. An important question for future research concerns the role of the central nervous system in this respect. Many areas in the hypothalamus and brain stem project to the intermediolateral column of the spinal cord, the motor nuclei of the sympathetic nervous system.[43] We want to investigate which areas in the central nervous system and which neurotransmitters and neuropeptides are involved in the adjustment of the sympathetic system during exercise. Preliminary results show that serotonergic and noradrenergic mechanisms in the hypothalamus might be involved. Serotonergic activity in the paraventricular area seems to increase selectively EPI output from the adrenal medulla and glucose output from the liver, whereas NOR output from the sympathetic nerve endings is suppressed.[44] Noradrenergic mechanisms in the paraventricular area seem to suppress selectively NOR output from the sympathetic nerve endings.[45]

Finally, the results of this study show that in the treatment of patients with α- and β-adrenergic agonists and antagonists, their influence on metabolism should be taken into account.

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