Enzymatic Adaptation to Physical Training under β-Blockade in the Rat

Evidence of a β_2-Adrenergic Mechanism in Skeletal Muscle

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Abstract

Nonselective and β_1-selective adrenergic antagonists were tested for their effects on enzymatic adaptation to exercise training in rats as follows: trained + placebo (TC); trained + propranolol (TP); trained + atenolol (TA); and corresponding sedentary groups, SC and SP. Trained rats ran 1 h/d at 26.8 m/min, 15% grade, 5 d/wk, 10 wk. Both β-antagonists were given at doses that decreased exercise heart rates by 25%. Training increased skeletal muscle citrate synthase, cytochrome *c* oxidase (Cyt-Ox), carnitine palmitoyltransferase (CPT), β-hydroxyacyl coenzyme A dehydrogenase, mitochondrial malate dehydrogenase (MDH), and alanine aminotransferase (ALT) activities significantly in the TC group, but not in TP. These enzyme activities, except Cyt-Ox and CPT, were also significantly increased in TA. Hepatic phosphoenolpyruvate carboxykinase activity did not alter with training or β-blockade. Fructose 1,6-bisphosphatase activity was lower in TC than in SC, but unchanged in TP or TA. Hepatic mitochondrial MDH and ALT activities increased with training only in TC. It is concluded that β_2-adrenergic mechanisms play an essential role in the training-induced enzymatic adaptation in skeletal muscle.

Introduction

Among the numerous physiologic changes occurring during prolonged exercise, the high level of sympathoadrenergic activity observed has received particular attention (1). Based upon the observation that chronic activation of the sympathoadrenergic system alone, as produced by cold acclimation, repeated heat exposure, or long-term injection of the β-adrenergic stimulant isoprenaline, could induce the activities of oxidative enzymes in skeletal muscle (2, 3), some physiologists believe that functional adrenoceptors are necessary for a metabolic adaptation of physical training. This hypothesis has received support from the observations that morphologic and enzymatic adaptation in heart and skeletal muscle did not take place either in sympathectomized rats (4), or in rats receiving a β-adrenoceptor antagonist, propranolol (5). However, other authors reported that sympathectomy did not influence the enzyme adaptation in skeletal muscle in rats (6) and that chronic β-blockade in clinical dosage did not prevent the conditioning effects of physical exercise in humans (7, 8).

This controversy is of significant clinical importance because most cardiac patients who take β-blocking agents are also physically active. Some studies demonstrated that the normal benefits of physical training, such as an increased maximal oxygen uptake ($\dot{V}O_2max$), an increased peak work capacity, and an enhanced endurance could be achieved despite drug administration (9, 10), whereas others questioned the conditioning effects of training under β-blockade (11, 12). Although it is generally believed that during exercise training, cardiovascular (CV)[1] functions are suppressed by the β-blocking drugs (13–16), whether or not the whole-body adpatational changes observed after training occur at the level of peripheral tissues has not been clearly resolved.

One possible reason for the discrepancy concerning the effect of β-adrenergic blockade on training adaptation is the different drug doses used by various authors. Because β-blocking drugs are competitive inhibitors of adrenoceptors (17), insufficient blockade could leave part of the receptors capable of binding with catecholamines, thereby eliciting adrenergic effects. Recently, there is evidence that the β-adrenergic receptor density is significantly higher in trained rat skeletal muscle as compared with that in sedentary animals (18). This mechanism could possibly compensate, at least in part, for the diminished catecholamine-receptor binding in the face of either adrenodemedullation or β-blockade, and provide required sympathoadrenergic stimulation to enzyme adaptation during training. Another problem in studying the role of the β-adrenergic system in training adaptation is the overall effects on the body of the widely used β-blocking drug, propranolol. The nonselective nature of propranolol could cause alterations of responses to exercise in various organs other than skeletal muscle. For example, changes of hormonal response to exercise or the free fatty acid (FFA) release from adipose tissue, could indirectly influence the skeletal muscle adaptation to training. Therefore, β_1-selective adrenergic blocking agents, such as atenolol or metoprolol, are preferred in order to differentiate the role of β_2-receptors existing in skeletal muscle. In addition, hepatic gluconeogenesis, which provides an exogenous fuel to skeletal muscle, i.e., blood glucose, in prolonged exercise is also regulated by catecholamine (19). Both β-adrenoceptor–mediated, cAMP-dependent mechanisms and α-ad-

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1. *Abbreviations used in this paper:* ALT, alanine aminotransferase; CPT, carnitine palmitoyltransferase; CS, citrate synthase; CV, cardiovascular; Cyt-Ox, cytochrome *c* oxidase; EHR, exercising heart rate; F-1,6-P_2ase, fructose 1,6-bisphosphatase; GPDH, α-glycerophosphate dehydrogenase; HADH, β-hydroxyacyl coenzyme A dehydrogenase; HR, heart rate; MDH, malate dehydrogenase; PEPCK, phosphoenolpyruvate carboxykinase; RHR, resting heart rate; SC, sedentary control; SP, sedentary propranolol; TA, trained atenolol; TC, trained control; TP, trained propranolol.

renoceptor–mediated, Ca^{2+}-dependent mechanisms are believed to play a role (20, 21). It would be of great importance to know whether the hepatic enzymes that regulate gluconeogenesis adapt to chronic training and to what extent they are influenced by β-blockade.

In the present study (22), we used both a nonselective β-blocker (propranolol) and a β_1-selective β-blocker (atenolol) to impose β-adrenoceptor blockade in the rat. The doses of both drugs were titrated to have maximal and equal potential in terms of the attenuation of exercising heart rate (EHR). Therefore, the hypothesis that a functional β-adrenergic system is required in the training-induced enzyme adaptation in skeletal muscle was tested; secondly, we investigated the influence of chronic physical exercise and β-adrenergic blockade on the activities of hepatic gluconeogenic enzymes and other related enzymes.

Methods

Animal care and training program. 30 male Sprague-Dawley rats (Sasco, Oregon, WI), aged 2 mo, body wt 250–275 g, were used in this experiment. Animals were housed two or three per cage in a temperature-controlled room (22°C) with a dark–light cycle of 12–12 h, and maintained with Purina rat Chow (Ralston-Purina Co., St. Louis, MO) and tap water ad libitum. On the day of arrival, the animals were assigned randomly into five groups: trained without drug or trained control (TC), trained with atenolol (TA), trained with propranolol (TP), sedentary control (SC), and sedentary with propranolol (SP). Food intake of the SC and SP groups was restricted to match the gain in body weight of their trained counterparts.

The exercise training was performed on two rodent motor-driven treadmills, 5 d/wk for 10 wk, at a speed of 26.8 m/min (1.0 mph). The first week's training sessions were 30 min at 0% grade. Thereafter, the duration of each work period and the slope of the treadmills were increased by 10 min and 5% per week, respectively. By the end of the fourth week, the animals were able to run on the treadmills for 60 min at 15% grade. This intensity of training was maintained for the following 6 wk. All training sessions were proceeded by a 5-min warm-up period at 15 m/min, 15% grade. To ensure that exercise training was performed effectively for all animals, training sessions were monitored continuously and carefully. The treadmills were equipped with an electrical shocking grid to provide exercise motivation to the animals. The dark–light cycle was adjusted such that all exercise training sessions were scheduled in darkness when the animals were in their active and feeding state. During the 1-h training sessions, propranolol-treated rats had some difficulty performing treadmill running in the first 3 wk. Under constant and careful monitoring and progressive training, these rats gradually adjusted to the work load and effectively finished 1-h running. After approximately 3–4 wk of training, all β-blocked rats could run on the treadmill as effectively as their unblocked counterparts without necessarily receiving more electrical shocking. To control the effect of routine handling, the control groups (SC and SP) also ran on the treadmill at 15 m/min, 0% slope for 5 min, scheduled at the same time of day as the training groups.

Administration of β-adrenergic blocking drugs. Groups TP and SP were started on a daily dose of propranolol 5 mg/kg body wt and group TA on atenolol 2.5 mg/kg. 2 wk after the onset of the training program when animals had been familiarized with treadmill running and daily handling, a titration experiment was conducted to examine the heart rate (HR) response to various drug doses. Six animals were randomly selected from the non-drug groups to participate in this experiment. Three sterilized brass electrodes were inserted subcutaneously on both sides of the chest and the back of the neck of the rat, which conducted the animal's cardiac electrical impulses by wire leads to an electrocardiograph and a multichannel electrocardiograph recorder. After the resting HR (RHR) was observed stable, the animals performed a progressive exercise test during which the EHR was recorded. The initial treadmill speed was 15 m/min and was then increased to 20 m/min at the fifth minute and 26.8 m/min at the ninth minute (grade remained constant at 15%). EHR was recorded at the end of the fourth minute of running at each speed. The HR response to exercise was similar among the six animals. 2 h after the exercise test when the RHR of the animals had been restored to their pretest levels, the six animals randomly received propranolol (Inderal) 10, 30, or 50 mg/kg body wt or atenolol (Tenomin) 5, 10, or 30 mg/kg body wt, respectively. The β-blocking drugs were dissolved in 1 ml of saline and injected intraperitoneally. 60 min after the drug administration, the previously described progressive exercise test was repeated and EHR was recorded. It was found that atenolol 10 mg/kg body wt and propranolol 30 mg/kg body wt reduced the EHR by the same percentage, i.e., approximately by 25% as compared with the predrug rate, while the animals were running at 26.8 m/min, 15% grade (same intensity as the training sessions). 30 mg/kg body wt atenolol or 50 mg/kg body wt propranolol, respectively, did not further attenuate the EHR. In addition, animals seemed to have difficulty tolerating these higher doses of drugs.

In the following 8 wk of the training sessions, the TA group received atenolol 10 mg/kg body wt, the TP and SP groups received propranolol 30 mg/kg body wt, 5 d/wk. Drugs were injected intraperitoneally approximately 30 min before each training session; the TC and SC groups received 1 ml saline (placebo). To further examine the effectiveness of the β-blockade in attenuating EHR, a week after drug administration (third week of the training), six animals were randomly selected from each of the trained groups and run on the treadmills for 1 h according to the training protocol. EHR was recorded every 5 min during the entire exercise period. The results are illustrated in Fig. 1. Both atenolol and propranolol significantly ($P < 0.01$) reduced the EHR by 25% as compared to the controls. There was no significant difference in EHR between the two drug-treated groups. This procedure was also periodically performed in the remaining 7 wk to ensure the effectiveness of the β-blockade.

Tissue preparation. All animals were killed 60 h after their last training sessions in a resting, fed, and nonmedicated state; the sedentary control animals were killed at the same time of the day. To minimize day-to-day variation, one animal from each group was sacrificed each day for 6 consecutive days.

After decapitation and exsanguination, the abdominal cavity of the rats was opened and the liver was excised and placed in an ice-cold

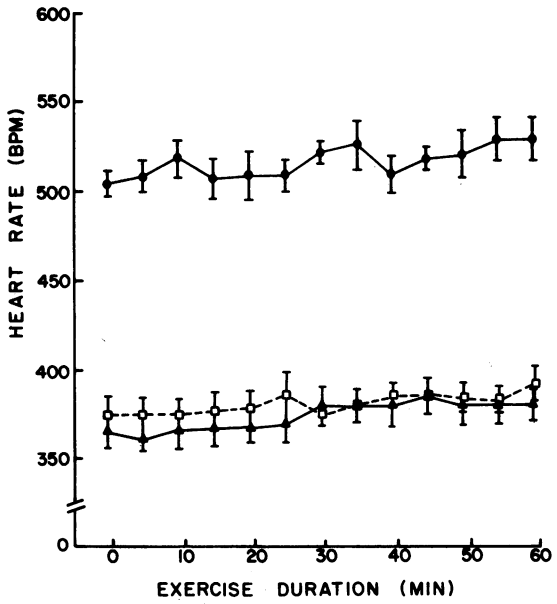

Figure 1. EHR responses during 1-h training session in the TP (▲), TA (□), and TC (●) rats. The differences in EHR between the drug groups and controls are statistically significant ($P < 0.01$). Each point represents data from six animals (mean±SE). EHR was recorded after a 5-min warm-up period at 15 m/min, 15% grade.

medium. The entire musculature of both hindlimbs was also quickly removed and placed in an appropriate medium. Liver mitochondria were prepared by the method of Johnson and Lardy (23) using Medium A containing 250 mM mannitol, 70 mM sucrose, and 1 mM EDTA (pH 7.4). The muscles were subsequently freed of extracellular fat and connective tissues, blotted, weighed, and placed in mitochondrial preparation medium consisting of 250 mM mannitol, 70 mM sucrose, 0.2% fatty acid-free albumin, and 13 U/ml collagenase (CLS II), pH 7.4. The volume of the medium was adjusted so that the weight/volume ratio was 1:10. The muscle slices were minced with scissors and homogenized with a Polytron (model PT-10, Brinkmann Instruments, Westbury, NY). The tissues were kept cool with ice during the homogenization. The homogenate was strained twice through one layer of medicine gauze to remove remaining fat and connective tissues. After centrifugation at 700 *g* for 10 min, the supernatant was centrifuged at 12,000 *g* for 10 min. The pellets were resuspended in medium A and centrifuged again at 12,000 *g* for 10 min. The mitochondrial pellets were stored frozen in 250 mM sucrose, 2 mM EDTA (pH 7.4) at −30°C until mitochondrial enzyme assays were performed.

The liver cytosolic enzymes were assayed in the supernatant fraction of a homogenate prepared in an ice-cold medium (10 mM Hepes, pH 7.4), and centrifuged at 700 *g* for 5 min to remove cell membranes, nuclei, and other debris. The supernatant was then centrifuged at 105,000 *g* for 60 min, and was stored frozen at −30°C until enzyme assays were performed.

Enzyme assays. The mitochondrial suspensions were frozen (dry ice-alcohol) and thawed three times to release the total enzyme activities. Skeletal muscle citrate synthase (CS, EC4.1.3.7) activity was determined spectrophotometrically at 25°C according to Shepherd and Garland (24); cytochrome *c* oxidase (Cty-Ox, EC1.9.3.1) was assayed at 37°C according to the method of Smith (25). The rate of oxidation of ferrocytochrome *c* was measured by following the decrease in the absorbancy of its α-band at 550 nm; mitochondrial malate dehydrogenase (MDH, EC 1.1.1.37) was measured at 30°C according to the method of Englard and Siegel (26); α-glycerophosphate dehydrogenase (GPDH, EC1.1.95.5) was assayed at 37°C by the method described by Wernette et al. (27); β-hydroxyacyl coenzyme A dehydrogenase (HADH, EC1.1.1.35) activity was measured at 25°C according to Essen et al. (28); carnitine palmitoyltransferase (CPT, EC2.3.1.21) activity was measured at 30°C by the isotope exchange method described by Norum (29) as modified by McGarry and Foster (30); alanine aminotransferase (ALT, EC2.6.1.2) activity was assayed at 30°C by the method of Bergmeyer and Bernt (31); phosphoenolpyruvate carboxykinase (PEPCK, EC4.1.1.49) was assayed at 25°C according to the method of Bentle and Lardy (32); and fructose 1,6-bisphosphatase (F-1,6-P_2ase, EC3.1.3.11) was assayed spectrophotometrically at 23°C according to Marcus et al. (33). Protein concentration in various tissue fractions was determined by the Biuret method, except for the CPT assay which was determined by the Bradford (34) method.

All chemicals, buffers, and enzymes were of the highest purity available. Propranolol was from Sigma Chemical Co., St. Louis, MO, and atenolol was from Stuart Pharmaceuticals, Wilmington, DE.

Statistical method. Statistical significance was tested by Student's *t* test. The tolerance of the type 1 error (α) level was set at 0.05.

Results

Body weight. Chronic administration of the nonselective β-blocking drug, propranolol, significantly decreased the body weight of only the trained animals. Although the rats had free access to food, the body weight of the TP group was 16% lighter ($P < 0.05$) than their non-drug counterparts TC, after 10 wk of administration of propranolol (Table I). In contrast, the β_1-selective blocker atenolol did not affect the growth rate, inasmuch as the body weight of TA was similar to that of TC. The body weight of SP also tended to be lower than SC but the difference was not statistically significant.

Table I. Body Weight of Various Groups of Rats before and after Training

Experimental group	1st week		10th week		
	Mean	SD	Mean	SD	*n*
	g	*g*	*g*	*g*	
TC	234	7	434	27	6
SC	233	8	418	39	6
TP	230	8	364*	41	6
SP	229	9	377	29	6
TA	232	10	413	49	6

* $P < 0.05$ (TP vs. TC).

Skeletal muscles. The specific activities of the skeletal muscle mitochondrial Krebs cycle enzyme CS and the enzyme of the respiratory chain, Cyt-Ox, were significantly higher in chronically trained rats than in the sedentary control group (Fig. 2). The average increase was 50% for CS ($P < 0.01$) and 38% ($P < 0.05$) for Cyt-Ox. The activities of these two mitochondrial enzymes in the TA group were also higher compared with those of SC, but statistically significantly only in the case of CS ($P < 0.01$). In contrast, there were no alterations in these two enzyme activities in the TP group compared with SP.

Skeletal muscle CPT and HADH were measured to assess the potential of the muscle to metabolize fatty acids (Fig. 3). After training, there was a significant increase in both CPT activity (+30%, $P < 0.05$) and HADH activity (+47%, $P < 0.001$). However, these enzyme adaptations were not observed in the

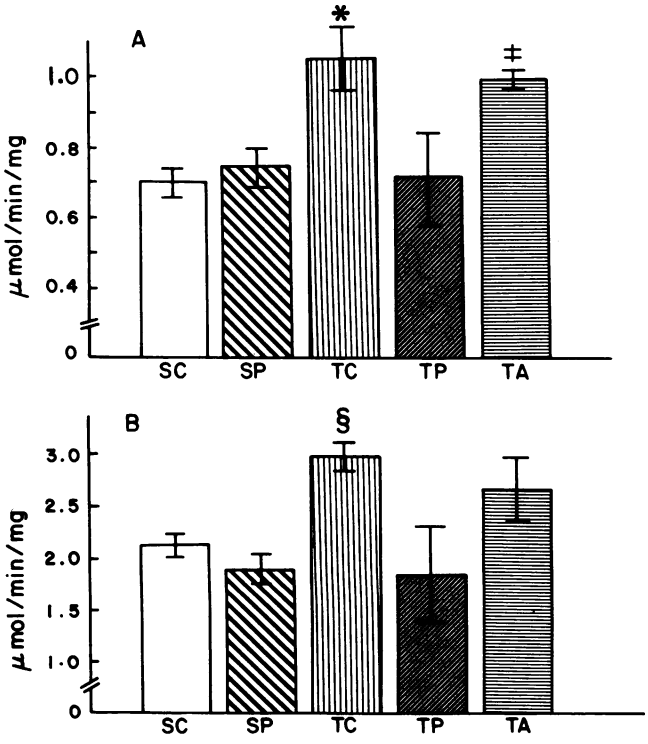

Figure 2. (*A*) Skeletal muscle citrate synthase and (*B*) cytochrome *c* oxidase specific activities (μmol/min/mg mitochondrial protein) in the SC, SP, TC, TP, and TA rats. Bars show standard error. *$P < 0.01$ (TC vs. SC); ‡$P < 0.05$ (TA vs. SC); §$P < 0.05$ (TC vs. SC).

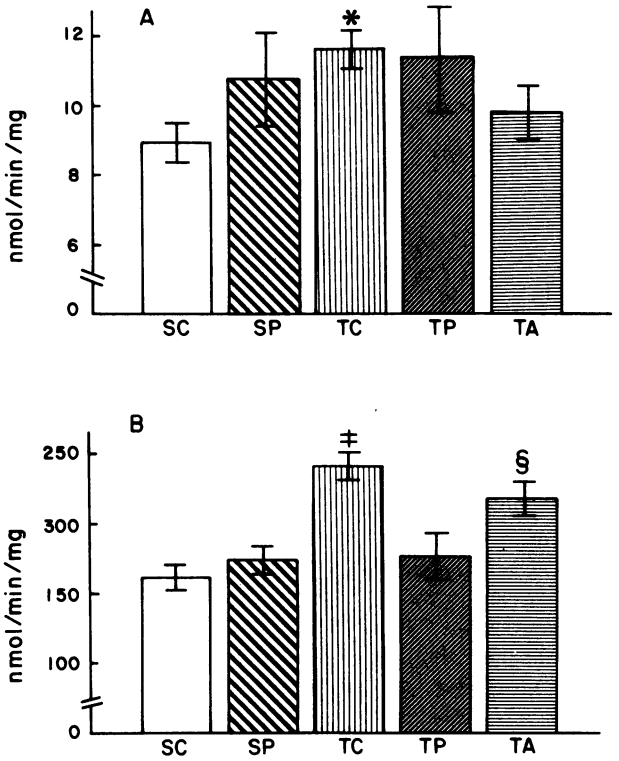

Figure 3. (*A*) Skeletal muscle CPT and (*B*) HADH specific activities (nmol/min/mg mitochondrial protein) in the various groups of rats as in Fig. 2. *$P < 0.05$; ‡$P < 0.001$ (TC vs. SC); §$P < 0.01$ (TA vs. SC).

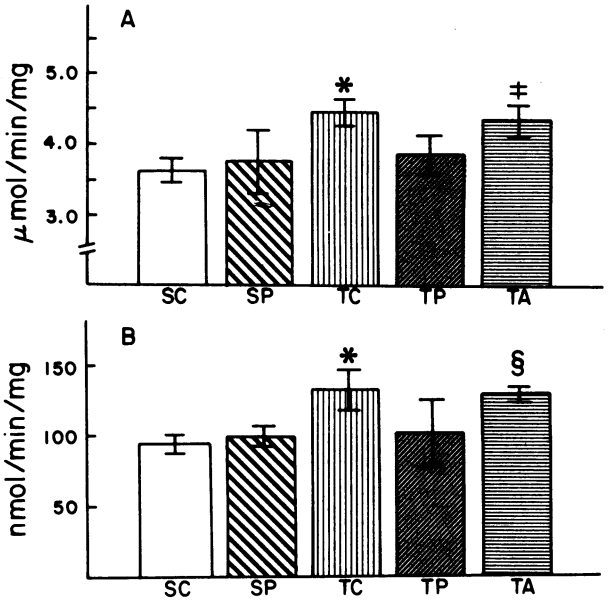

Figure 4. (*A*) Skeletal muscle mitochondrial MDH (μmol/min/mg mitochondrial protein) and (*B*) ALT (nmol/min/mg mitochondrial protein) specific activities in various groups of rats as in Fig. 2. *$P < 0.05$ (TC vs. SC); ‡$P < 0.05$; §$P < 0.01$ (TA vs. SC).

propranolol-treated rats. In the TA group, the HADH activity was also significantly higher (+32%, $P < 0.01$) than in SC, whereas the CPT activity was not significantly altered.

Skeletal muscle mitochondrial MDH, which participates in the tricarboxylic acid cycle and in the malate-aspartate shuttle to transfer reducing equivalents, was increased significantly with training both in the TC group (+26%, $P < 0.05$) and in the TA group (+20%, $P < 0.05$), but not in the TP group (Fig. 4 *A*). GPDH activity, which reflects the capacity of the α-glycerophosphate shuttle, was not significantly altered by training, or by either β-blocking drug (data not shown).

Alanine aminotransferase, which was assayed to reveal the potential of the glucose-alanine cycle (35), adapted to training with a 43% ($P < 0.05$) increased activity (Fig. 4 *B*). This adaptation was also observed in the atenolol-treated rats (+40%, $P < 0.01$) but not in the propranolol-treated rats.

Liver. Hepatic PEPCK activity varied from 5.88 to 6.94 nmol/min/mg protein among the five groups of rats. Neither training or β-blockade affected the hepatic PEPCK activity ($P > 0.05$) (data not shown). However, for another key gluconeogenic enzyme, F-1,6-P_2ase activity was significantly lower in the TC group (−27%, $P < 0.05$) (Fig. 5 *A*). F-1,6-P_2ase activity in TP was not significantly different from the respective control, SP group. Atenolol prevented the decrease in F-1,6-P_2ase activity during exercise training.

Hepatic mitochondrial MDH activity was significantly higher in the TC group than in SC group (+38%, $P < 0.001$) (Fig. 5 *B*). Both propranolol and atenolol eliminated the training-induced adaptation of this enzyme. Similar to what was found in the skeletal muscle, the hepatic GPDH activity was not significantly altered by either training or β-blockade (data not shown).

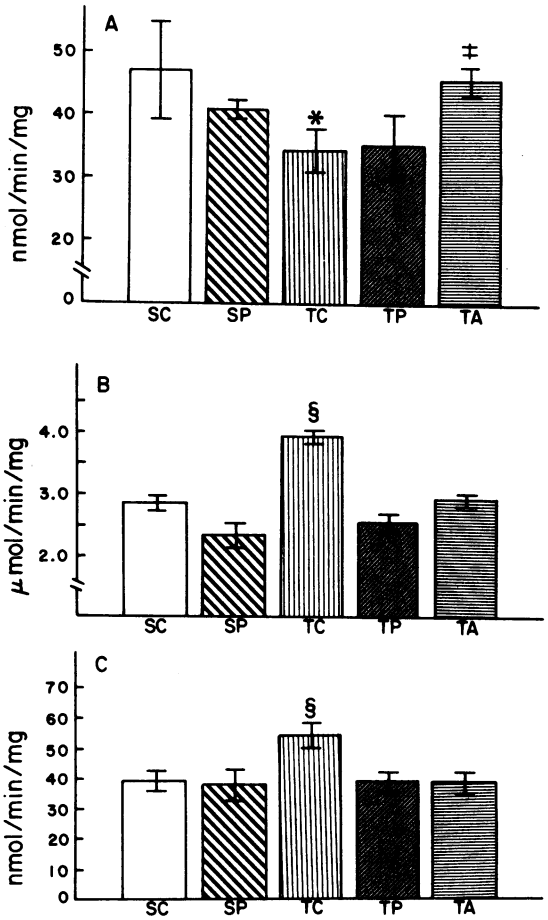

Figure 5. (*A*) Hepatic F-1,6-P_2ase (nmol/min/mg cytosolic protein), (*B*) mitochondrial MDH (μmol/min/mg) mitochondrial protein), and (*C*) mitochondrial ALT (nmol/min/mg mitochondrial protein) specific activities in various groups of rats as in Fig. 2. *$P < 0.05$ (TC vs. SC); ‡$P < 0.05$ (TA vs. TC); §$P < 0.01$ (TC vs. SC).

Training increased the hepatic mitochondrial ALT activity in the TC animals by ~37% ($P < 0.01$), however, it had no effect when propranolol or atenolol were administered (Fig. 5 *C*).

Discussion

Body weight. The significantly lower body weight in the TP group of rats was not expected, as none of the studies involving β-blockade in human cardiac patients showed a significant effect of long-term drug administration on body weight. In a previous experiment, we examined the effects of 8-wk administration of propranolol (30 mg/kg body wt, i.p.) on body composition in the sedentary rat (manuscript submitted) and found no difference in bw between the β-blocked and unblocked animals. However, rats in the present experiment were subjected to an intense exercise training program. The well-known effects of propranolol on lipolysis and glycogenolysis might have limited the utilization of fat and muscle glycogen; therefore rats had to break down body protein as energy substrate. Consequently, growth of these animals might be impaired.

Skeletal muscle. Although the influence of chronic endurance training upon the skeletal muscle oxidative capacity has been known for decades (36–38), the mechanism(s) that cause(s) these changes are not clear. One observation that has been recognized by many physiologists is that prolonged exercise of substantial intensity is associated with a high level of sympathetic activity and blood catecholamines (1). Since Harri and Voltola (3) reported that repeated isoprenaline injection resulted in significant increase in mitochondrial oxidative enzymes in rat skeletal muscle, various studies have been done to examine the possibility that the enzymatic adaptation in skeletal muscle in response to physical training is actually mediated by a sympathoadrenergic stimulation associated with exercise rather than the exercise per se. Liang et al. (39) found that infusion of dobutamine, a synthetic catecholamine, into dogs 2 h/d for 5 wk produced CV and metabolic conditioning effects similar to those induced by exercise training. Later, Harri (5) reported that a daily dose of propranolol 10 mg/kg body wt given to a group of rats involved in a 7-wk running program severely attenuated the increase in the activities of a number of skeletal muscle enzymes with training. He concluded that a functional sympathetic nervous system was required for an efficient adaptation of muscle metabolism to physical training.

However, this conclusion has received more challenges than support. Juhlin-Dannfelt (7), for example, claimed that chronic β-blockade did not prevent the conditioning effects of physical exercise based upon the observation that the heart weight and skeletal muscle enzyme activities in chronically trained rats increased despite β-blockade. However, the β-blocking drug (propranolol, 16 mg/100 g body wt, daily) was given in the food and the effectiveness of the blockade was not reported. Svedenhag et al. (8) examined the effect of propranolol (160 mg/d) on metabolic and enzymatic adaptation to training in healthy humans and found only a borderline difference between the drug-treated and control subjects. Furthermore, Henriksson et al. (6) found no significant difference in the activities of the mitochondrial enzyme CS, succinate dehydrogenase, Cyt-Ox, and HADH between the intact rats and either adrenodemedullated rats or the sympathectomized rats after a swimming training program. Therefore, they concluded that neither adrenomedullary hormones nor sympathetic nerves are prerequisites for the training-induced adaptation of muscle enzymes.

In the face of this apparent controversy regarding the role of the sympathoadrenergic system in training adaptation, we thought there were two limitations in methodologies of the previous studies. First, a wide variation of β-antagonist dose levels were used by various authors. In human subjects, side effects prevented the investigators from applying higher dosage. Thus, in these subjects (either animals or humans) who underwent a training program, there was no ensurance that the influence of the sympathoadrenergic system was maximally blocked. Secondly, almost all studies previously mentioned used the nonselective β-antagonist, propranolol. Although propranolol has been the most common β-blocking drug used in clinical practice, the nonselective nature of this drug makes it difficult to draw conclusions regarding its effects on the training adaptation of peripheral tissues such as skeletal muscle.

In the present investigation, we applied both the β_1-selective and nonselective antagonists to rats which were subjected to an intense 10-wk training program. It was found that the normally observed, training-induced muscle enzyme adaptations such as CS, Cyt-Ox, HADH, MDH, and ALT were almost completely eliminated by propranolol but not by atenolol. Because the doses of both β-blockers were titrated to suppress the EHR to the same maximal extent, we were assured that the contribution of the CV system to training adaptation in peripheral tissues was ruled out, inasmuch as it is well known that the main effect of β-blockade on the CV system during exercise is to reduce EHR (15). One possible explanation of the differences in enzymatic adaptations between the propranolol- and atenolol-treated rats is the supply of FFA to the skeletal muscle during exercise. It is known that the adrenoceptors in rat adipose tissue are predominately of the β_1 type, although β_2-receptors also exist (40). Therefore, the β_1-selective blocker might inhibit lipolysis to a lesser extent than the nonselective blocker. Some investigators reported lower plasma FFA levels in exercising humans under the nonselective β-blocker propranolol than under the β_1-selective metroprolol (41). However, others showed that when EHR was equally reduced by nonselective and β_1-selective blockade, plasma glycerol, and FFA levels were also reduced to the same extent (42). The reason for this apparent discrepancy is not clear. However, it is conceivable that these human subjects exercised at a much lower intensity as compared with our animals. The β-blocking drug doses were also considerably lower. It is possible that much higher doses are required to inhibit lipolysis completely. We did not measure the plasma FFA concentration in the rats. However, it is not likely that the dramatic differences in enzyme activities we observed between the propranolol- and atenolol-treated rats after training were merely due to the differences in plasma FFA levels during exercise.

Recently, Williams et al. (18) reported that the β-adrenergic receptor density in rat skeletal muscle was significantly increased after 10 wk of treadmill running and this increase was positively correlated with muscle succinate dehydrogenase activity. This finding might be of significant biological importance in that it suggested that the spare receptors which are not occupied by catecholamine may increase with training. Therefore, a higher dose of β-blocking drug is required to inhibit a given biochemical response to catecholamine than before training. This mechanism probably explains why even moderately high doses of propranolol have failed to prevent the training-induced enzymatic adaptation in muscle. In contrast, in the present investigation, the sufficiently high dose of propranolol (30 mg/kg body wt) has probably adequately antagonized the increased β-adrenoceptors and achieved

maximal blockade. Taking all these findings into consideration, we postulate that the sympathoadrenergic stimulation plays an essential and permissive role in eliciting enzymatic adaptations in skeletal muscle. The binding of catecholamine to a minimal number of β_2-adrenergic receptors may provide a sufficient level of adenylate cyclase to allow, along with other signals, elicitation of adaptational changes normally seen with chronic exercise training.

The differential effects of propranolol and atenolol on the training induction of HADH, but not CPT, are rather interesting. Although both enzymes are involved in fatty acid oxidation in muscle mitochondria, HADH is generally considered the rate-limiting step. Since the plasma FFA level is lower during exercise under both nonselective and β_1-selective blockade (7, 40), data in this study seem to indicate that there are other factors than plasma FFA that contribute to the training adaptation of HADH. For example, intramuscular triglyceride lipase activity has been reported to increase with training (43), and catecholamine seems to activate this lipase by a cyclic AMP-dependent β-adrenergic mechanism (44). Thus, the endogenous source of fatty acid might provide substrate for mitochondrial β-oxidation under the β_1-selective blockade, but not under the nonselective blockade.

The adaptational increase in ALT activity to physical training was also prevented by propranolol. We first postulated that the glucose–alanine cycle might become an increasingly important pathway in the face of a diminished supply of FFA to exercising muscle under β-blockade, and thus the mitochondrial ALT might adapt to this situation by increasing total activity. However, this was not the case. The diminished availability of pyruvate in muscle under nonselective β-blockade, which is known to impair alanine synthesis (45), might have limited the operation of the glucose–alanine cycle. On the other hand, the ALT activity in the atenolol treated rats increased to almost the same extent as in the non-drug controls after training, indicating that the glucose-alanine cycle may play a compensatory role under β_1-selective blockade.

Liver. Gluconeogenesis in perfused liver and in isolated hepatocytes is stimulated by glucagon and by catecholamine (46, 47). During prolonged exercise, the plasma concentrations of glucagon and catecholamine are elevated (19) and along with the increased levels of blood gluconeogenic precursors, the hepatic gluconeogenic enzymes, namely PEPCK and F-1,6-P_2ase, are also reported to be activated (48). Therefore, after chronic exercise training, one might expect an adaptational change in these enzymes. However, we found no difference in the total activities of hepatic PEPCK between the chronically trained and untrained rats. A literature search indicates that little is known concerning adaptation of the hepatic gluconeogenic enzymes to exercise training. One of the important reasons is that liver is a dynamic organ and the hepatic gluconeogenic enzymes have a relatively short half-life. Lardy et al. (49) pointed out that although long-term adaptation can occur to PEPCK, activity of this enzyme in response to hormonal signals to produce blood glucose is too rapid to be accounted for by an increased amount of enzyme protein. Surprisingly, another rate limiting enzyme in hepatic gluconeogenesis, F-1,6-P_2ase was significantly decreased in total activity after training. F-1,6-P_2ase is regulated by complicated hormonal and metabolic factors including the concentration of its substrate fructose 1,6-bisphosphate (F-1,6-P_2) and of its two inhibitors, AMP and fructose 2,6-bisphosphate (F-2,6-P_2) (47). There is evidence that during prolonged exercise F-1,6-P_2ase is activated by epinephrine or glucagon via cyclic AMP-dependent phosphorylation of F-6-P-2 kinase which decreases the level of F-2,6-P_2 (48). Releasing the inhibition of F-1,6-P_2ase would diminish the amount of enzyme protein required for conducting gluconeogenesis and the mechanisms that regulate the synthesis of this protein are probably attenuated.

In liver, whether the catecholamine stimulated-glycogenolysis and gluconeogenesis are mediated by α- or β-adrenergic receptors varies with species as well as sex and age of the experimental animals. In adult male rats, hepatic α-receptors play a predominant role (50, 51). Furthermore, the regulation of gluconeogenesis at the site of PEPCK has been claimed to be predominately under the influence of the α-adrenergic mechanism via Ca^{2+} (49, 52). Results in this investigation showing that PEPCK activity was not affected by β-adrenergic blockade in the chronically trained rats supported these hypotheses and seemed to indicate that hepatic gluconeogenesis is not under β-adrenergic control. However, it has been reported by various investigators that hypoglycemia occurs during prolonged exercise and is more severe under β-blockade, especially under the nonselective β-blocker propranolol (7, 41, 53). One explanation for this phenomenon is that the lowered plasma FFA levels, and possibly also the inhibited muscle glycogenolysis, with propranolol makes the exercising muscle depend more upon blood glucose. Apparently, hepatic glucose production cannot cope with the peripheral glucose uptake during prolonged exercise under β-blockade (53). In light of the results from the present investigation, it is clear that failure to replenish blood glucose under β-blockade was not due to the lower hepatic enzyme capacity, but rather, to the impaired peripheral supply of gluconeogenic precursors, such as pyruvate, lactate, and alanine (53).

We found that, after training, there was a significant increase in hepatic mitochondrial MDH and ALT activities, whereas the activity of the hepatic mitochondrial GPDH was not changed. The increased activity of MDH could potentially facilitate the formation of malate from oxalacetate in mitochondria and hence the flux of pyruvate to phosphoenolpyruvate. In contrast, the unchanged GPDH activity after chronic training indicated that either the glycerophosphate shuttle was not biologically as important as the malate-aspartate shuttle in rat liver, or that Ca^{2+}, released under the influence of α_1-adrenergic stimulation, increased the activity of the enzyme sufficiently to meet the demand for enhanced transport of reducing equivalents between cytosol and mitochondria (27). The increase in both the total hepatic and skeletal muscle mitochondrial ALT activity after 10 wk of training is in accord with the hypothesis that the glucose–alanine cycle plays an important role in supplying blood glucose during prolonged exercise (35).

In contrast to the findings in skeletal muscle, we did not see a differential effect of propranolol and atenolol on the training-induced adaptation of hepatic MDH and ALT activities, as both β-blockers eliminated the training-induced adaptation of these enzymes. Little information is available concerning the influence of catecholamine and β-blockade on the regulation of these two enzymes. It is most likely that the decreased enzyme activities were due to the decreased levels of the blood-borne substrates under β-blockade.

Summary. The main findings of the present investigation were that the enzymatic adaptations in rat skeletal muscle observed after intensive endurance training could be prevented by a sufficiently high dose of the nonselective β-blocking agent propranolol, but not by the β_1-selective blocking agent, atenolol at doses that exerted an equal effect on EHR. Our results support

the hypothesis that stimulation of the β_2-adrenergic receptors located in skeletal muscle is required for a significant enzymatic adaptation to endurance training.

The data indicate that hepatic enzymes functioning solely in gluconeogenesis do not adapt to chronic physical training by increasing total activities, as is the case of skeletal muscle mitochondrial enzymes. Chronic training did increase the activities of hepatic enzymes indirectly involved in gluconeogenesis, and β-blockade impaired the training adaptation of these enzymes, indicating that liver plays an important role in the interorgan cooperation under metabolic stress.

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