PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **180**, 527–532 (1985)

Effect of Contractile Activity on Rat Skeletal Muscle β-Adrenoceptor Properties (42212)

RONALD D. FELL, FLORENCE H. LIZZO, PETER CERVONI, AND DAVID L. CRANDALL

Exercise Physiology Laboratory, University of Louisville, Louisville, Kentucky 40292, and Department of Cardiovascular Biological Research, American Cyanamid Company, Medical Research Division Lederle Laboratories, Pearl River, New York, 10965

Abstract. The effect of fiber type and endurance exercise training on skeletal muscle β-adrenoceptor properties were assessed using a direct radioligand binding technique. Six separate muscles, composed of a variety of different fiber types, were examined in treadmill trained and sedentary rats. In trained animals, sarcolemmal preparations from heart and slow twitch soleus muscle exhibited a significantly greater receptor concentration than membranes from white fast twitch glycolytic fibers of the vastus lateralis. No significant changes were observed between trained and sedentary rat muscle β-adrenoceptor density (β_{max}, fmole/mg protein) or affinity (K_d, nM) within each muscle type, despite significantly increased myocardial/body weight ratios and skeletal muscle enzyme adaptations associated with the exercise program. These results suggest that muscle β-adrenoceptor properties may be influenced in part by the motor nerve innervation to that muscle, and are further discussed with respect to a possible relationship between exercise intensity and receptor regulation.

Endurance exercise is associated with an increased oxidative capacity in skeletal muscle, which may be mediated in part by changes in the autonomic regulation of specific metabolic processes (1, 2). Since many of these processes, including aspects of the regulation of substrate supply, are mediated through β-adrenergic stimulation, the possibility exists that the membrane-bound receptor may be an important regulatory site affected by training.

In a recent investigation, endurance treadmill training was observed to induce an increased β-adrenoceptor density in two separate hindlimb skeletal muscles of the rat (3). β-Adrenoceptor density (β_{max}) was also assessed with respect to oxidative capacity within fiber type, with the conclusion that highly oxidative slow twitch soleus muscle exhibited a greater receptor concentration than the mixed fiber type fast twitch gastrocnemius (3). Taken together, these results suggested that, regardless of the method of induction, an increased skeletal muscle oxidative capacity was directly related to an increased β_{max}. This conclusion, however, conflicts with an earlier report in which β-adrenoceptor concentration was greater in fast twitch extensor digitorum longus muscle than in slow twitch soleus (4). Because no apparent explanation exists for the discrepancy between these studies, additional experiments examining a greater variety of muscles composed of differing proportions of known fiber types would certainly aid in the interpretation of the relationship between oxidative capacity and receptor density.

To further assess the regulation of β-adrenoceptor density in skeletal muscle, a direct radioligand binding technique has been performed in sarcolemmal preparations from five different skeletal muscles of sedentary and exercised rats. Muscles selected represented a wide range of the three fiber types found in rat skeletal muscle. Together with values obtained for heart muscle, these data provide additional insight into the relationship between the physiological function of a specific muscle type and its β-adrenoceptor density.

Methods. Male Sprague–Dawley rats weighing 225–250 g were maintained on a diet of Purina Chow and water. The animals were housed individually in a temperature-controlled room (20 ± 1°C) with a 12-hr light: dark cycle. Half the animals were trained to run on a motor-driven treadmill until they were able to run 5 days/week at 22 m/min up a 15% grade for a period of 30 min. Animals remained on this training protocol for 12 weeks. The rest of the rats served as sedentary cage control animals. Twenty-four hours after the last exercise bout, animals were anesthe-

0037-9727/85 $1.50

tized with sodium pentobarbital (6 mg/100 g body wt) and muscles from each hindlimb were dissected out and clamp-frozen with tongs cooled in liquid nitrogen. The muscles frozen were the soleus, which consists predominantly of slow twitch red fibers; the plantaris, which consists of approximately 53% fast-twitch red, 41% fast-twitch white, and 6% slow-twitch red fibers; the superficial portion of the vastus lateralis, which is made up of fast-twitch white fibers; the deep portion of the vastus lateralis, which consists of fast-twitch red fibers; and the gastrocnemius which is made up of 37% fast-twitch red, 58% white fibers and 5% slow-twitch red fibers (5). The thoracic cavity was opened, and the heart was removed and quickly trimmed free of atria, great vessels, and fat. The ventricles were blotted and weighed prior to being clamp-frozen. All tissues were kept at −80°C until analyzed. In a number of animals, the plantaris muscle (not frozen) was minced, homogenized in 300 m*M* sucrose, 10 m*M* Tris–HCl, pH 7.2, and 2 m*M* EDTA, and used for measurement of respiratory capacity in a Gilson differential respirometer at 30°C in the presence of nonlimiting concentrations of Pi and ADP, with pyruvate plus malate as substrates (6).

Membrane preparation and receptor assay. Preparation of membranes for identification of myocardial β-adrenoceptors proceeded according to the method of Baker *et al.* (7), employing modifications previously described by our laboratory (8). Membrane protein recovered from one heart each was sufficient for the assay procedure. Preparation of membranes for identification of skeletal muscle β-adrenoceptors generally proceeded according to the method used for myocardial tissue (7, 8), with the exception of two modifications. The first modification involved the use of different numbers of individual skeletal muscles for each assay dependent upon muscle mass, e.g., the smaller soleus required pooling of six muscles for one assay preparation. On the morning of the experiment, frozen muscles were removed from −85°C storage, thawed, minced, and homogenized in 10 ml of cold Tris buffer [Tris(hydroxymethyl)aminomethane–HCl, 10 m*M*, pH 8.0] with a Polytron PT-10 homogenizer set at speed 6 for 45 sec. Each homogenate was diluted with 1 *M* KCl and placed on ice for 10 min, which has been shown to facilitate the denaturing of contractile protein and thus increase specific binding (7). At this point, a second procedural modification was performed to facilitate the removal of connective tissue and improve the recovery of skeletal muscle membrane protein. The modification involved the transfer of the aforementioned homogenate to a 50-ml plastic syringe in which the beveled needle attachment had been cut away, leaving only the barrel and plunger. Nylon mesh (149 μm; Spectrum Medical Industries, Inc., Los Angeles, Calif.) was attached to the open end of the barrel, followed by the application of slight pressure to the plunger. The resulting filtrate was then centrifuged at 4°C at 48,000*g* for 10 min. Pellets were resuspended in 20 ml of buffer, and again resedimented by centrifugation, ultimately yielding the skeletal muscle membrane preparation.

Analysis of membrane protein by the method of Lowry *et al.* (9) indicated that a 5–10× increase in recovery of total membrane protein was obtained by including the syringe filtration procedure, when compared directly to preparation of the membrane by centrifugation only. Additionally, the recovery of membrane protein in the final preparation was verified by the presence of a membrane marker enzyme, 5′-nucleotidase (10).

Aliquots of cardiac and skeletal muscle membranes containing 325 ± 25 μg protein were incubated in triplicate at seven different concentrations of [^{3}H]dihydroalprenolol (DHA) ranging from 0.07 to 5 n*M* with or without D,L-propranolol HCl as described previously (8). The concentration of membrane protein used per assay remained constant for control and exercise-trained rats. Following the incubation period, the membranes were separated from the medium by a Whatman GF/C glass fiber filter and Millipore sampling manifold. Specific binding associated with the membrane preparation was assessed with a Beckman LSC 6800 liquid scintillation counter, and was approximately 60% of total binding. Dissociation constants (K_d) and maximum receptor number (β_{max}) were calculated by the method of Scatchard (11).

Muscles from control rats were always assayed the same day as tissue obtained from exercise-trained rats. Also a linear response between the concentration of membrane pro-

tein (50–500 μg) and specifically bound [^{3}H]DHA was observed when the concentration of [^{3}H]DHA was kept constant.

[^{3}H]DHA has been shown to bind β-adrenergic receptors in a rapid, reversible, saturable, and stereospecific manner, and has been used to identify β-adrenergic receptor binding sites in a variety of tissues (12, 13, 14). The purity of [^{3}H]DHA (New England Nuclear, Lot 1912-098) used was monitored on a regular basis by thin-layer chromatography, and at all times remained above 96% pure. The high specific activity of ligand used for these experiments (104.8 Ci/mmole) allowed the identification of small quantities of receptor protein.

Statistical analysis. Comparisons between treatment (trained vs sedentary) body weights, heart weights, and maximum oxygen uptake capacity (plantaris muscles) were made using Student's *t* test. Analysis of variance followed by Newman–Keul's post hoc analysis was used to statistically evaluate differences in β-adrenoceptor binding properties (β_{max} and K_d) between and within treatments. $P < 0.05$ was chosen as the level of significance.

Results. Common to endurance-exercise training in male rats is a voluntary reduction in food intake (15, 16). Reduced caloric consumption was manifested in this study by significantly ($P < 0.001$) lower body weights of trained animals (Table I). There was no significant difference between absolute heart weights of sedentary and trained rats; however, when heart weight was expressed as a ratio of body weight, training was observed to result in a significant elevation of this ratio (Table I).

The maximum capacity to oxidize malate and pyruvate during state 3 respiration was measured in plantaris (P) muscle homogenates and the oxygen uptake data are shown in Table II. The treadmill training protocol used was intense enough to induce a significant increase

TABLE I. BODY WEIGHTS AND HEART WEIGHTS AFTER TREADMILL TRAINING

Group	*N*	Body wt (g)	Heart wt (g)	Heart wt/body wt (mg/g)
Sedentary	18	375 ± 5.95	975 ± 16.8	2.608 ± 0.038
Trained	18	322 ± 11.0*	956 ± 22.6	3.014 ± 0.072*

Note. Values are means ± SEM.
* Significantly different from sedentary values at $P < 0.001$.

TABLE II. MALATE-PYRUVATE OXIDATION IN PLANTARIS MUSCLES AFTER EXERCISE TRAINING

Group	*N*	Oxygen uptake (μl O_2/g wet wt/min)
Sedentary	7	42.8 ± 3.75
Trained	7	50.4 ± 4.03*

Note. Values are means ± SEM.
* Significantly different from sedentary value at $P < 0.01$.

in the maximum oxidative capacity within the exercised skeletal muscles. Collectively the data in Tables I and II suggests that the training protocol was significantly rigorous to cause both cardiovascular and skeletal muscle adaptations.

The β-adrenoceptor concentrations (β_{max}) and receptor affinity, as measured by K_d from sedentary and trained rat muscles are shown in Table III. While β_{max} exhibited a tendency to increase in three skeletal muscles, the endurance training protocol used in this investigation resulted in no changes in either receptor concentration or binding affinity. Highly oxidative myocardial tissue had the greatest number of β-adrenoceptors but this concentration was not significantly different from slow twitch soleus (S) muscle values which are also oxidative. The β-adrenoceptor density of the S was not significantly different from the density in P, gastrocnemius (G), or the red portion of the vastus lateralis (VL), but was greater than the density in the white VL. The P, G, red, and white VL did not have significantly different β_{max} values.

Within each muscle type, binding affinity (K_d) was not affected by exercise training. Within each treatment, heart and soleus muscle exhibited the lowest K_d, and as with receptor density, these values were not statistically different from each other. In trained rats, the binding affinity in white muscle was significantly higher from those values obtained from heart and soleus muscle.

Discussion. Skeletal muscle β-adrenoceptors have previously been identified by direct ligand binding studies (17, 18), and have been reported to be influenced by several experimental conditions (1). Chronic exposure of skeletal muscles to elevated catecholamine concentrations has been shown to decrease the

TABLE III. EFFECT OF EXERCISE AND MUSCLE TYPE ON β-ADRENERGIC RECEPTOR DENSITY AND AFFINITY

Muscle	Sedentary			Trained		
	N	β_{max}	K_d	N	β_{max}	K_d
Heart (1)[a]	6	45.1 ± 3.25*	1.12 ± 0.14	5	46.6 ± 5.05**	1.21 ± 0.16†
Soleus (6)	4	28.3 ± 2.94	0.53 ± 0.27***	6	37.3 ± 3.74†	0.46 ± 0.22†
Gastrocnemius (1)	4	22.7 ± 4.42	3.04 ± 1.24	4	30.9 ± 5.54	2.51 ± 0.86
Plantaris (4)	5	29.2 ± 4.72	1.82 ± 0.44	5	27.0 ± 4.22	2.40 ± 0.87
Red (2)	4	19.2 ± 4.22	2.21 ± 0.46	4	26.0 ± 3.42	2.81 ± 0.67
White (1)	5	19.3 ± 4.76	2.74 ± 0.29	5	17.7 ± 3.63	4.09 ± 0.80

Note. Values are means ± SEM, β_{max} = fmole/mg protein, K_d = nM.

[a] Number of muscles required per assay in parentheses.

* Significantly different from gastrocnemius, white and red values within same treatment.

** Significantly different from plantaris, red and white values within same treatment.

*** Significantly different from gastrocnemius values within same treatment.

† Significantly different from white values within same treatment.

density (β_{max}) of β-adrenoceptors (19), suggesting that catecholamines play a direct role in regulating β-adrenoceptor concentration. The loss of tissue catecholamine sensitivity, due to a lower β-adrenoceptor density, may in part explain the attenuated epinephrine induced glycogenolysis observed in an earlier study in which skeletal muscles from rats were chronically exposed to high levels of epinephrine (20).

Results of these experiments suggest that there are multiple factors involved in controlling muscle β-adrenoceptor properties and in turn the muscle's sensitivity to adrenergic stimulation. In addition to circulating levels of catecholamines mentioned above, it has also been demonstrated that the muscle's motor nerve can influence the concentration of muscle fiber β-adrenoceptors (14). When rat gastrocnemius was denervated, the β-adrenoceptor concentration increased. This finding is of particular interest since motor innervation of skeletal muscles is known to be cholinergic rather than adrenergic and raises the question of whether or not the muscle fiber recruitment pattern, as dictated by the innervating motor neuron threshold, controls to some extent the β-adrenergic receptor properties of the muscle. Data from the present investigation support such a hypothesis. The skeletal muscle fibers recruited to contract most frequently (slow twitch soleus) have a higher β-adrenoceptor density than muscle which is recruited to contract only during very intense types of exercise (white VL). Further support for this finding comes from a recent study which examined β-adrenoceptor properties in only slow twitch oxidative fibers from the soleus and mixed fast twitch fibers (FOG and FG) from rat gastrocnemius muscles (3). These investigators found a positive relationship between β-adrenoceptor concentration and oxidative capacity of the muscle. The present investigation examined a number of muscles with a wider range of fiber type composition, particularly the fast twitch glycolytic white and fast twitch oxidative red portions of the VL. Since the rat fast twitch red VL has a higher oxidative capacity than the slow twitch fibers of the soleus muscle (21), the current receptor data suggests a correlation between the level of recruitment and β-adrenoceptor density in skeletal muscle as opposed to oxidative capacity.

If recruitment of muscle contractile activity influences β-adrenoceptor properties as suggested, then endurance training should have increased the concentration of muscle (heart and skeletal) β-adrenoceptors. On the other hand, daily bouts of exercise have been shown to increase the concentration of circulating catecholamines (22), and as previously discussed, exposure to elevated catecholamines would decrease the number of muscle β-adrenoceptors. It is therefore possible that these antagonistic factors, elevated catecholamines and increased recruitment (contractile activity), have resulted in a lack of change in β-adrenoceptor concentration in the present study.

The treadmill exercise used in this study was of moderate intensity, and despite a significant training effect, evidenced by both myocardial/

body weight changes and an elevation in the maximum oxidative capacity of a mixed fast twitch muscle (plantaris), these adaptations were small when compared to studies which have used more rigorous training protocols (6). It is possible that a more intense training procedure would elicit a higher level of muscle contractile activity and increase the β-adrenoceptor concentration. Evidence for this suggestion was presented in a recent study which used a longer treadmill training regimen (3). These investigators found an elevated β-adrenoceptor density with a more intense treadmill training procedure and yet no changes in skeletal muscle receptor number were observed with a moderate swimming exercise program. Since catecholamine responses to the same absolute work bout are lower after training (1), a higher level of physical activity may attenuate the catecholamine response to a greater degree. A lower catecholamine response may then allow the increased contractile activity during daily more intense exercise training to facilitate an increase in β-adrenoceptors.

Absolute receptor values for any particular muscle fiber type vary widely between laboratories, however, with some reports indicating a skeletal muscle β_{max} of 10–20 times that of cardiac muscle (4, 19). Since β-adrenoceptor concentration varies with respect to sympathetic nervous system innervation (23) and because cardiac muscle is innervated to a greater extent than skeletal muscle (24, 25), these reports appear paradoxical. The values we report for skeletal muscle β_{max} are similar to, yet slightly less than those for cardiac receptors, and agree with similar reports from other laboratories with respect to both cardiac and skeletal muscle (18, 24). The discrepancy between our values and the aforementioned higher values may be due in part to methodological differences. Our methodology is unique with respect to the use of a high-specific-activity ligand, a preparative procedure involving contractile protein digestion that increases specific binding (7), and an additional filtration that resulted in removal of much of the connective tissue.

In conclusion, the physiological role and regulation of β-adrenoceptors remains largely undefined, particularly during exercise. It has been shown that β-adrenergic blockade does not effect muscle glycogenolysis during exercise (26). Since catecholamines can activate glycogenolysis in skeletal muscle by the cAMP-dependent protein kinase mechanism (27) the β-adrenoceptor complex may be playing a role in initiating substrate availability (glycogenolysis) prior to or in anticipation of an exercise. Results of the present investigation suggest that muscle recruitment patterns may play a role in the regulation of β-adrenoceptor concentration in skeletal muscle. In addition, both contractile activity and catecholamine hormone exposure may influence receptor properties in an antagonistic manner. Further studies involving a combination of contractile activity and hormonal influences would be valuable in further determining the mechanisms of β-adrenoceptor control in skeletal muscle.

This work was supported in part by a grant from the American Heart Association, Kentucky Affiliate.

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Received February 25, 1985. P.S.E.B.M. 1985, Vol. 180.
Accepted July 24, 1985.