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β-ADRENERGIC RECEPTORS IN INNERVATED AND DENERVATED SKELETAL MUSCLE

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Summary

In order to determine if the development of β-adrenergic receptors may explain the catecholamine evoked contracture of denervated mammalian skeletal muscle, the binding capacities and dissociation constants of β-adrenergic receptors of innervated and denervated rat skeletal muscle membrane preparations were determined by using [^{3}H]dihydroalprenolol. The dissociation constants of [^{3}H]dihydroalprenolol binding to innervated and denervated muscle microsomal suspensions were similar. The maximal number of binding sites increased from 27 pmol/g protein to 85 pmol/g protein following 25 days denervation. These results suggest that motor nerve may be involved in part, in the regulation of β-adrenergic receptors in skeletal muscle membrane preparations.

Chronically denervated mammalian skeletal muscles fibrillate spontaneously and exhibit a tone which is dependent on the frequency of the spontaneous fibrillation [1—4]. Catecholamines have been shown to produce contractures in chronically denervated mammalian muscle both in vivo [5—7] and in vitro [8—10] and this increase in tension produced by catecholamines in denervated muscle is accompanied by an increase in fibrillation [5, 11]. In innervated muscles catecholamines do not cause contractures but indirectly affect evoked twitch tension through both a pre- and post-synaptic action [12, 13]. The development of catecholamine sensitivity in chronically denervated muscles can be prevented by injecting the animals intraperitoneally with the protein synthesis inhibitor actinomycin D within the first two days after motor nerve section [10]. Following denervation, mammalian skeletal muscle also produces a contracture in the presence of acetylcholine and it has been demonstrated that this response is the result of the development of cholinergic receptors over the entire muscle fiber surface, a process believed to be linked to

protein synthesis [14—17]. Therefore, the present experiments have been designated to investigate whether a similar development of β-adrenergic receptors can explain the catecholamine-evoked contracture of denervated mammalian skeletal muscle.

The properties and density of β-adrenergic receptors on the microsomal suspensions of innervated and denervated rat skeletal muscles were determined by using a potent β-adrenergic receptor antagonist [^{3}H]dihydroalprenolol as a radioactive ligand which has been shown to bind specifically to β-adrenergic receptors [18—20]. Male Sprague-Dawley rats (120—160 g) were killed by decapitation, and gastrocnemius muscle was rapidly removed and placed on ice. All the connective tissues and nerves were removed with scissors, and the muscle was minced and homogenized with a Brinkmann Polytron in 20 volumes of ice-cold 0.05 M Tris (pH 8.0 at 25°C) and centrifuged at 49 000 x *g* for 15 min. The pellet was homogenized in the same buffer and centrifuged as before. The pellet was finally suspended in 50 volumes of Tris buffer (pH 8.0 at 25°C) and used fresh for the binding assay.

[^{3}H]Dihydroalprenolol (32 Ci/mmol) was purchased from New England Nuclear Corporation, Boston, Massachusetts, and the purity of the compound was checked by thin-layer chromatography, as described before [20]. [^{3}H]Dihydroalprenolol binding was determined at 23°C by adding 0.97 ml of the above particulate suspension, 0.5 to 4 nM [^{3}H]dihydroalprenolol, and sufficient water to bring the final volume of the reaction mixture to 1 ml. After a 20-min incubation, the reaction mixture was filtered under reduced pressure through Whatman glass fibers (GF/B). The filters were rinsed four times with 4 ml of ice-cold Tris buffer to remove most of the unbound radioactive ligand. The filter papers were dried and placed in a counting vial containing 10 ml of Scintiverse (Fisher Co.) and counted in a Packard Tri-Carb liquid scintillation spectrometer (Model 3380) at 30% efficiency. Corrections were made for nonspecific accumulation of radioactivity by assaying a parallel incubation containing a large excess (100 μM) of noradrenaline. Specific binding, defined as the difference between total and nonspecific radioactivity was 60—70% of the total binding. The procedure for the iodination of α-bungarotoxin and for measuring its specific binding to particulate fractions was similar to that described for the iodination and binding of [^{125}I]nerve growth factor to sympathetic ganglion membranes [21].

The specific binding of [^{3}H]dihydroalprenolol to a particulate fraction derived from rat gastrocnemius muscle was found to have properties similar to those that would be expected for binding to β-adrenergic receptors in vitro. The binding was reversible, saturable, of high affinity, and stereospecific; and it was inhibited by appropriate β-adrenergic receptor ligands. Ablation of the sciatic nerve markedly modified the specific binding of [^{3}H]dihydroalprenolol to muscle membrane preparations (Table I). When the concentration of [^{3}H]-dihydroalprenolol was 1 nM, the specific binding to innervated gastrocnemius muscle preparations was found to be 8.2 pmol/g protein; this increased to 12.6 and 32.2 pmol/g protein 4 days and 25 days after denervation, respectively (Table I). These results clearly demonstrate that denervation of the gastrocnemius muscle increases the specific [^{3}H]dihydroalprenolol binding to muscle particulate fractions. This increase may be due to alterations in ap-

TABLE I

SPECIFIC [^{3}H]DIHYDROALPRENOLOL AND [^{125}I]α-BUNGAROTOXIN BINDING TO INNERVATED AND DENERVATED SKELETAL MUSCLE

Denervation was carried out 4 or 25 days before sacrificing the animals by the removal of 1 cm of the sciatic nerve close to its point of entry into the muscle. A sham operation was performed on the opposite leg, which served as control. The procedure for the preparation of the particulate fraction and measurement of specific binding of [^{3}H]dihydroalprenolol (1 nM) and [^{125}I]α-bungarotoxin to membrane fractions are described in the text. Results are means ± 1 S.E.M. and were obtained from 8 animals in control and 4 animals each in 4 days denervated and 25 days denervated gastrocnemius muscles. The average total weights of muscles were: control, 1.9 g; 4 days denervated, 1.7 g; and 25 days denervated, 0.92 g. The specific binding of [^{125}I]α-bungarotoxin (93 Ci/g) to innervated and 25 days denervated muscle particulate fraction were 4915 ± 416 and 28 654 ± 2138 cpm/mg protein, respectively.

Concentration of [^{3}H]dihydroalprenolol (nM)	Specific [^{3}H] dihydroalprenolol binding		
	Innervated	4 days denervated (pmol/g protein)	25 days denervated
1	8.2 ± 0.07	12.6 ± 0.13	32.2 ± 0.29
4	18.2 ± 0.19	29.0 ± 0.31	59.6 ± 0.54

parent affinity of [^{3}H]dihydroalprenolol to motor nerve denervated muscle preparations or to changes in the number of β-adrenergic receptors on muscle particulate fractions induced by interrupting neuromuscular transmission. The apparent affinities of [^{3}H]dihydroalprenolol for β-adrenergic receptors and the density of β-adrenergic receptors in the sciatic nerve innervated and denervated gastrocnemius muscle membrane fraction were determined by measuring the specific binding of various concentrations of [^{3}H]dihydroalprenolol and analyzing the data by the method of Scatchard [22] (Table II). The results are consistent with the idea that there is only a single class of high affinity binding sites. The dissociation constant of [^{3}H]dihydroalprenolol binding to motor nerve innervated muscle particulate fractions was found to be 1.76 nM. This value appears to be similar to the dissociation constant of [^{3}H]dihydroalprenolol binding to β-adrenergic receptors derived from other tissues, such as rat brain (1.3 nM) [20], frog erythrocyte (3.4 nM) [23] and dog heart (7 nM) [24]. Denervation of the gastrocnemius muscle did not change the dissociation constant of [^{3}H]dihydroalprenolol binding to skeletal muscle membrane fractions (Table II). The maximal number of specific binding sites of [^{3}H]dihydroalprenolol to innervated gastrocnemius muscle membrane preparations was 27 pmol/g protein (Table II). A similar concentration of β-adrenergic receptors has been reported for rat liver (39 pmol/g) [25], but this value is different from that found in rat brain (290 pmol/g) [26], rat heart

TABLE II

SCATCHARD ANALYSIS OF SPECIFIC [^{3}H]DIHYDROALPRENOLOL BINDING TO INNERVATED AND DENERVATED SKELETAL MUSCLE

The dissociation constant of [^{3}H]dihydroalprenolol and the density of binding sites were determined by Scatchard analysis [22]. The procedures for the preparation of the particulate fraction and measurement of specific binding of [^{3}H]dihydroalprenolol to membrane fractions are described in the text. Each value is the mean ± standard error of independent determinations on preparations from 4 to 8 animals (numbers shown in parentheses).

Muscle	Dissociation constant (nM)	Density of receptors (pmol/g protein)
Innervated	1.76 ± 0.10 (8)	27 ± 3.9 (8)
25 days denervated	1.63 ± 0.11 (4)	85 ± 6.8 (4)

(240 pmol/g) [27], and frog erythrocyte (250 pmol/g) [28]. Since some of these studies were done with a more purified membrane fraction, differences in densities of β-adrenergic receptor in different tissues and species may be related to the purity of particulate fractions. The most striking feature of the results shown in Table II is the three-fold increase in the density of [^{3}H]dihydroalprenolol binding to muscle membrane fraction 25 days after denervation. These observations would suggest that the development of catecholamine-induced contractures in chronically denervated mammalian skeletal muscle may be due to an increase in the density of β-adrenergic receptors on the muscle plasma membrane.

Although it is possible that the development of catecholamine-evoked contractures and increased density of β-adrenergic receptors may be two unrelated changes on the skeletal muscle cell surface following denervation, the present observations support the idea that the concentration of β-adrenergic receptors on the mammalian skeletal muscle particulate fraction is regulated by the motor nerve innervating the skeletal muscle. Recent results from several laboratories indicate that ablation of pre-synaptic catecholaminergic neurons causes an increase in density of β-adrenergic receptors [26, 29—31] at the post-synaptic sites. These results have led to the hypothesis that β-adrenergic catecholamines are able to regulate catecholamine sensitivity of tissues in vivo by regulating the number of properties of the β-adrenergic receptor binding sites [32]. Since motor nerves are not pre-synaptic neurons to β-adrenergic receptors on the surface of skeletal muscle, it is unlikely that noradrenaline alone regulates catecholamine sensitivity of tissues in vivo. It is possible that other factors released by catecholaminergic pre-synaptic neurons may participate with or without noradrenaline in the regulation of β-adrenergic receptor densities at the post-synaptic sites. Furthermore, some hormones may have "permissive" effects in the regulation of β-adrenergic receptor concentration [25]. Nevertheless, the present observations clearly show that, at least on the mammalian skeletal muscle surface, the β-adrenergic receptor density is not regulated by the concentration of noradrenaline in vivo.

In conclusion, surgical denervation of rat gastrocnemius muscle caused a three-fold increase in the density of β-adrenergic receptors in muscle particulate fraction without affecting their affinity for dihydroalprenolol. The results may provide a molecular basis for catecholamine evoked contractures in chronically denervated mammalian skeletal muscles. In addition, this observation provides evidence against the hypothesis that the concentration of noradrenaline is responsible in the regulation of β-adrenergic receptor density on the surface of mammalian skeletal muscles.

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