Sodium Channel and Sodium Pump in Normal and Pathological Muscles from Patients with Myotonic Muscular Dystrophy and Lower Motor Neuron Impairment

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ABSTRACT Two sodium transport systems have been analyzed in this work: the voltage-sensitive sodium channel and the (Na^+, K^+) ATPase pump. The sodium channel has been studied using a tritiated derivative of tetrodotoxin; the sodium pump has been studied using tritiated ouabain. Properties of interaction of tritiated tetrodotoxin and of tritiated ouabain with their respective receptors were observed in normal human skeletal muscle and in muscles of patients with myotonic muscular dystrophy and with lower motor neuron impairment.

Levels of sodium pump and of sodium channels were measured at different stages of membrane purification. Microsomal fractions of normal human muscle have maximal binding capacities for tetrodotoxin of 230 fmol/mg of protein and of 7.4 pmol/mg of protein for ouabain.

Dissociation constant for the complexes formed by the tetrodotoxin derivative and by ouabain with their respective receptors were 0.52 nM and 0.55 μM, respectively.

In muscles from patients with myotonic muscular dystrophy, the maximal binding capacity for tetrodotoxin, i.e., the number of Na^+ channels was found to be very similar to that found for normal muscle. The maximal binding capacity for ouabain, i.e., the number of Na^+ pumps was three- to sixfold lower than in normal muscle. Dissociation constants for the complexes formed with the tetrodotoxin derivative and with ouabain were the same as for normal muscle.

In muscles from patients with lower motor nerve impairment, the maximal binding capacities for tetrodotoxin and for ouabain were twice as high as in normal muscle. Again, dissociation constants for the complexes formed with the tetrodotoxin derivative and with ouabain were nearly unchanged as compared with normal muscle.

These results suggest that sodium transport systems involved in the generation of action potentials and/or in the regulation of the resting potential are altered both in myotonic muscular dystrophy and in lower motor neuron impairment.

INTRODUCTION

Myotonic disorders are inherited diseases whose clinical manifestation is delayed relaxation of the muscles after voluntary contraction. Myotonia also includes electrophysiological signs such as increased excitability, which has been shown by electromyography examination as a tendency to fire characteristic trains of repetitive action potentials in response to wild contraction or to direct electrical or mechanical stimulation.

Myotonia in man is commonly associated with muscular dystrophy and called myotonic muscular dystrophy (MyD)[1] or Steinert's disease. But it is also present

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[1] *Abbreviations used in this paper*: B_{max}, maximal binding capacity; H_0, purified muscle homogenates; K_D, dissociation constants; MyD, myotonic muscular dystrophy; STX, saxitoxin; [^{3}H]en-TTX, tritiated ethylenediamine tetrodotoxin; TSE, Tris-HCl, sucrose, and EDTA buffer; TTX, tetrodotoxin.

as the cardinal sign in nondystrophic forms such as myotonia congenita, or Thomsen's disease, and paramyotonia congenita (for syndrome classification, see ref. 1). All these disorders appear to be due to an abnormality of the muscle itself as they persist after section or blocking of the motor nerve and after curarization (2). Most experimental and clinical data suggest that human myotonia arises from genetically induced structural and functional alterations of muscle cell membranes. However, the specific defect has not yet been defined.

Nearly all of the relevant research related to myotonia has been carried out so far using experimental animal models, especially goats, with a hereditary myotonia that resembles autosomal dominant myotonia congenita of man in inheritance pattern, involvement of the muscle fiber membrane, and loss of membrane chloride conductance (3–5). The widespread organ involvement, apparent absence of circulating myotonic toxin, failure of any neurogenic or vascular theory to explain membrane abnormality also support in the case of MyD a primary defect of membrane proteins and/or lipids.

Another distinct aspect of muscle pathology is human neurogenic muscular atrophy, which occurs in lower motor neuron impairment such as motor neuron disease, disorder of motor nerve roots, and disorder of peripheral motor or mixed nerves (for syndrome classification see ref. 6). In all these diseased states, the muscular wasting is due to defective muscle innervation rather than an intrinsic affection of the tissue. Accordingly, morphological and electrophysiological data show that structural and functional deficiencies of muscle are linked to an alteration of neural influence. As indicated by electrophysiological analysis, specific abnormalities of the excitable properties of muscle fibers can be found in response to modification in acetylcholine discharge (7). This latter discharge, initiated by nerve impulse, controls the propagation of action potential by changing the ionic permeability of muscle membranes.

The generation of an action potential during excitation requires rapid sequential changes in membrane conductances of Na^+ and K^+ ions (8) that depend on the opening and closing of the respective channels (9). When the action potential has passed, the cell is left with a disturbed ion distribution that is restored by the enzyme (Na^+, K^+)ATPase (10).

We analyze in this paper the comparative properties of two important components of the Na^+ pathway through the muscle membrane, i.e., the voltage-sensitive Na^+ channel and the (Na^+, K^+)ATPase, in normal and pathological human muscle.

The levels of the Na^+ pump in normal and pathological muscle were measured using [^{3}H]ouabain, a cardiac glycoside that binds very specifically to the phosphorylated form of the enzyme (10–13). Densities of sodium channels were measured using a highly radioactive tetrodotoxin derivative, the synthesis of which has been recently described (14). Tetrodotoxin is one of the toxins specific for the fast Na^+ conductance in excitable tissues; it blocks Na^+ passage through the channel (15, 16).

METHODS

Muscle biopsies. Samples (0.1–0.5 g) of human muscle were obtained by open biopsy on limb after informed consent. They were immediately frozen in isopentane chilled in liquid nitrogen and kept at −80°C until use. A part was processed as previously described (17) for histochemical examination. The other part served for binding studies.

Normal subjects showed no specific clinical features, electromyographical profiles, morphological and histoenzymological muscular structures were normal.

The main clinical and laboratory characteristics of patients with myotonic dystrophy and lower motor neuron impairment are presented in Tables I and II.

Muscle fractionation for binding assays. Three different types of muscle preparations have been used: (*a*) crude homogenates (Hg_0), (*b*) purified homogenates (H_0) partly deprived of contractile proteins by extraction at high ionic strength, (*c*) a microsomal fraction (P_3) enriched in plasmatic membranes by suitable fractionation procedures.

Crude homogenates were obtained as follows: Biopsied samples of human muscle were thawed and washed to remove blood with an ice-cold 20-mM Tris-HCl buffer at pH 7.5 containing 0.25 M sucrose and 1 mM EDTA (TSE buffer). Muscle samples were then mixed with 10 vol of the TSE buffer and homogenized at setting 5 with a polytron homogenizer PT 10 S (Brinkmann Instruments, Westbury, N. Y.) using three 5-s bursts separated by 30-s pauses. Crude homogenates were then filtered through four layers of cheesecloth and used directly for binding assays.

Preparations of purified homogenates (H_0) and microsomal fractions (P_3) were carried out according to Desnuelle et al. (manuscript in preparation). Briefly, samples of human muscle were washed in an ice-cold buffer at pH 7.4 (buffer 1) containing 50 mM triethanolamine and 0.8 M KCl, and homogenized in 4 vol of the same buffer with a polytron apparatus at setting 6 using one 5-s burst.

Homogenates were then centrifuged at 20,000 *g* for 10 min. Pellets were resuspended in 1 vol of buffer 1 with a Potter-Elvejhem homogenizer and recentrifuged under the same conditions. New pellets were resuspended in 1 vol of an ice-cold buffer at pH 7.4 containing 50 mM triethanolamine and 10 mM $NaHCO_3$ (buffer 2) at pH 7.4 with the Potter homogenizer to obtain the purified homogenate H_0. H_0 was centrifuged at 20,000 *g* for 10 min. The supernatant S_1 was discarded and the pellet P_1 was washed once with buffer 2. After washing, pellet P_1 was suspended in 1 vol of buffer 2 with the polytron apparatus (setting 6: three 30-s bursts) and centrifuged at 2,000 *g* for 20 min. The supernatant S_2 was then centrifuged at 100,000 *g* for 1 h. The pellet P_3 corresponds to the microsomal fraction. It was diluted to a concentration of 1 mg protein/ml in a 20-mM Tris-HCl buffer at pH 7.4 containing 50 mM choline chloride, and used directly for binding assays. Further fractionation to isolate external membranes of a higher purity was not possible because of the small amount of normal or patho-

TABLE I
Clinical and Laboratory Data on the Patients with MyD

Patient	Age	Sex	Clinical finding: Family	Distal weakness	Distal dystrophy	Myotonia	Cataract	Frontal balding	Serum enzymes	Electromyography electrical mytonia	Histological finding: Muscle biopsied	Increased nucleation	Internal nuclei	Ring fiber	Type I fiber atrophy
1	40	M	+	−	−	+	−	−	N	+	Flexor carpi radialis	+	+	+	+
2	43	M	−	+	+	+	−	+	N	+	Gastrocnemius	+	+	+	+
3	28	M	Unknown	+	+	+	+	+	N	+	Peroneus longus	+	+	+	−
4	45	F	+	+	+	+	+	−	N	+	Peroneus longus	+	+	+	−
5	54	F	Unknown	+	+	+	−	−	N	+	Quadriceps	+	+	−	+
6	32	M	−	+	−	+	−	+	N	+	Deltoid	+	+	−	+

Histological signs noted (increased nucleation, internal nuclei, ring fibers, type I fiber atrophy), are known to be frequently observed in human MyD.

logical muscle available. Protein contents were determined using Hartree's method (18) with bovine serum albumin as a standard.

Binding assays. Measurements of the amount of Na^+ channels were determined using a tetrodotoxin derivative made by grafting tritiated ethylenediamine to tetrodotoxin (TTX) (14). Binding assays with this derivative [^{3}H]en-TTX were carried out in a choline chloride medium as previously

TABLE II
Clinical and Laboratory Data on the Patients with Lower Neural Impairment

Patient	Age	Sex	Clinical finding	Etiology*	EMG: Denervation§	EMG: MNCV‡	Muscle biopsied	Histochemical finding: Group atrophy	Fiber type atrophy	Target fiber
1	68	F	Polyneuropathy lower and upper limb	SLE	+	D	Gastrocnemius	+	+	+
2	52	F	Polyneuropathy lower limb	Toxic	+	D	Gastrocnemius	+	+	+
3	63	M	Mononeuropathy tibialis post.	PN	+	D	Gastrocnemius	+	+	−
4	69	M	Sensory motor polyneuropathy	PN	+	D	Gastrocnemius	+	+	−
5	42	M	Sensory motor polyneuropathy	Alcoholic (toxic)	+	D	Peroneus longus	+	+	+
6	58	M	Mononevritis multiplex	Multiple Myeloma	+	D	Flexor carpi radialis	+	+	+
7	36	M	Mononevritis multiplex	Toxic	ND	ND	Peroneus longus	+	+	−
8	50	M	Distal muscular atrophy	ALS	+	N	Quadriceps	+	+	−
9	50	M	Spastic paraplegia	Familial spastic paraplegia	+	N	Gastrocnemius	+	+	−
10	65	F	Distal muscular atrophy	ALS	+	N	Deltoid	+	+	−
11	49	M	Distal muscular atrophy	ALS	+	N	Peroneus longus	+	+	−
12	60	M	Distrophy muscular atrophy	ALS	+	N	Deltoid	+	+	+

* SLE, systemiae lupus erythemateous; PN, periarteritis nodosa; ALS, amyotrophic lateral sclerosis.

‡ MNCV, motor nerve conduction velocity; D, decreased; N, normal.

§ Denervation: positive sharp waves at rest with reduced number of functional motor units during activity with or without fibrillation potentials—motor unit potentials increased in duration and amplitude.

described elsewhere (19). Briefly, 0.1–0.5 mg of protein of crude muscle homogenate, purified homogenate or microsomes were incubated at 4°C in 1 ml of the standard incubation medium (20 mM Tris buffer containing 50 mM choline chloride at pH 7.5) with appropriate concentrations of radioligand in the absence (total binding) or in the presence (nonspecific binding) of 5 μM TTX. After 20 min each incubation was stopped by filtering in duplicate two aliquots of 0.4 ml through prewetted GF/B glass fiber filters over vacuum. Filters were rinsed twice with ice-cold buffer and counted with Picofluor 30 (Packard Instrument Co., Inc., Downers Grove, Ill.). Aliquots of 0.1 ml were taken in parallel to determine the total radioligand concentration. Specific [^{3}H]en-TTX binding was determined from the difference between the radioactivity determined in the absence and in the presence of 5 μM native TTX as described above.

Binding assays with [^{3}H]ouabain were carried out as follows: 0.1 to 0.5 mg of protein of crude muscle homogenate were equilibrated in 0.25 ml of a 50-mM triethanolamine buffer at pH 7.4 containing 0.1 M NaCl, 5 mM $MgCl_2$, 2 mM ATP with various concentrations of [^{3}H]ouabain in the absence or in the presence of 1 mM cold ouabain. After an incubation of 1 h at 25°C, the reaction was stopped by filtering, in duplicate, aliquots of 0.1 ml through prewetted GF/B glass fiber filters (Whatman Inc., Clifton, N. J.) positioned over a vacuum Millipore filter flask apparatus (Millipore Co., Bedford, Mass.). Filters were rinsed twice with 5 ml of the ice-cold buffer that had served in the incubation. Aliquots of 0.04 ml were taken in parallel to determine the total radioligand concentration. [^{3}H]ouabain that remained bound to muscle membranes on the filter was extracted by vigorous shaking with 8 ml of Picofluor 30 (Packard Instrument Co.) as scintillator. Radioactivity was measured using a Packard 2450 scintillation counter. As for [^{3}H]en-TTX binding experiments, the free ligand concentration was calculated by subtracting the amount of [^{3}H]ouabain bound to muscle membranes from the total radioligand concentration. Specific [^{3}H]ouabain binding was determined from the bound radioactivity measured in the absence of cold ouabain and the bound radioactivity measured in the presence of 1 mM native ouabain (20). Binding experiments using purified muscle homogenates (H_0) or the microsomal pellet P_3 were carried out using the same procedure.

Analysis of data. [^{3}H]en-TTX and [^{3}H]ouabain binding data were analyzed according to Atkins (21) using a Wang Laboratories, Tewksbury, Mass., 2200 calculator to fit experimental points with theoretical curves. This fitting procedure provided Hill coefficients, maximal binding capacities, values of equilibrium dissociation constants (K_D) and IC_{50} values, i.e., the concentration of the compound that inhibits binding by 50% under the peculiar experimental conditions. The overall apparent dissociation constant $K_{0.5}$ of the inhibition for [^{3}H]en-TTX binding was calculated from the equation

$$K_{0.5} = \frac{IC_{50}}{1 + \frac{[[^3H]\text{en-TTX}]_f}{K_D}}, \quad (1)$$

where $[[^3H]\text{en-TTX}]_f$ is the free concentration of radioligand at half-dissociation and K_D the equilibrium dissociation constant for [^{3}H]en-TTX binding determined by direct binding assay.

Chemicals and drugs. [^{3}H]en-TTX was synthesized according to Chicheportiche et al. (14). This preparation of [^{3}H]en-TTX had a specific radioactivity of 27 Ci/mmol and a radiochemical purity of 90%. [^{3}H]ouabain was obtained from NEN Europe (Dreieich, West Germany). Citrate-free tetrodotoxin was purchased from Sankyo Chemical Co. (Tokyo, Japan), ouabain and ATP from Sigma Chemical Co. (St. Louis, Mo.). Saxitoxin was obtained from the Food and Drug Administration (Washington, D. C.).

RESULTS

[^{3}H]en-TTX and [^{3}H]ouabain binding to normal human muscle. Binding curves for [^{3}H]en-TTX and [^{3}H]ouabain were obtained with crude homogenates Hg_0, with the purified homogenates H_0 and with microsomes (Fig. 1). Muscle samples were obtained from eight normal subjects: three from quadriceps, three from gastrocnemius, one from tibialis anterior, and one from biceps. Experiments on crude homogenates were carried out on individual homogenates prepared from each normal muscle sample. Experiments with the purified homogenate fraction H_0 and with microsomes required more material and it was then necessary to pool the eight different normal muscle samples.

Typical [^{3}H]en-TTX binding curves to crude homogenates of normal muscles are represented in Fig. 2A. The Scatchard plot describing the specific binding (Fig. 2B) is linear and therefore indicates that there is a single family of binding sites and allows for the determination of the maximal binding capacity (B_{max}) and of the dissociation constant, K_D, of the [^{3}H]en-TTX-receptor complex. B_{max} values found with the eight different crude homogenates varied from 25 to 32 fmol/mg of protein. The average B_{max} (Table III)

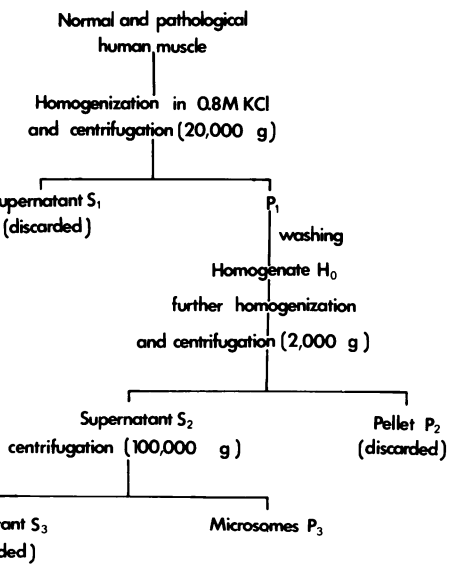

FIGURE 1 Schematic pathway of the fractionation of normal and pathological human skeletal muscle. H_0: purified homogenates with low amounts of contractile proteins. P_3: microsomal fraction with mixed population of external, mitochondrial and endoplasmic reticular membrane vesicles.

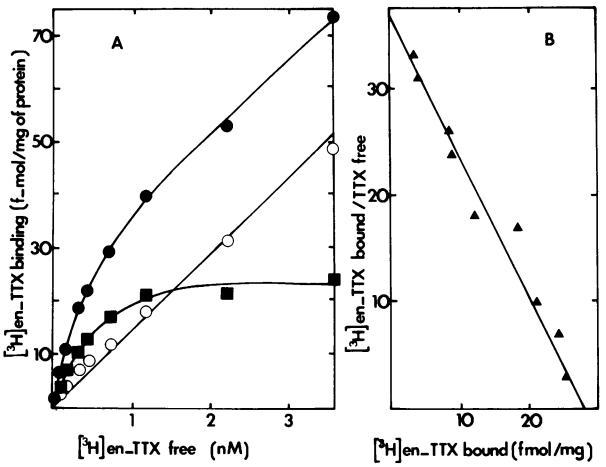

FIGURE 2 Binding of [³H]en-TTX to crude homogenates of normal human skeletal muscle at 4°C. Protein concentration was 0.5 mg/ml. (A) Total [³H]en-TTX binding (●) and nonspecific binding (○) were determined in parallel in the absence and in the presence of 5 μM TTX. Specific binding (■) is reported, calculated from total and nonspecific binding. In this typical experiment, K_D and B_{max} values of 0.50 nM and 25 fmol/mg of protein were obtained, respectively. (B) A Scatchard analysis of the specific [³H]en-TTX binding data. K_D = 0.52 nM and B_{max} = 28 fmol/mg of protein. The ordinate is bound over free ligand (milliliters per gram of protein) and the abscissa is bound ligand (femtomoles per milligram of protein).

was therefore 28±4 fmol/mg of protein. The maximum binding capacities were 60 and 230 fmol/mg of protein in homogenate H_0 and in the microsomal fraction, respectively. K_D were 0.52, 0.55, and 0.62 nM for the crude homogenate, the purified homogenate, and the microsomal fraction, respectively.

Unlabeled tetrodotoxin and saxitoxin antagonize [³H]en-TTX binding from crude homogenate of normal muscle (Fig. 3). Dissociation constants were calculated as reported in Methods by using Eq. 1. They are 1.0 and 0.85 nM for TTX and saxitoxin (STX), respectively.

Typical results obtained for [³H]ouabain are presented in Fig. 4A, B. Here, again, Scatchard analysis of the binding data indicated a single family of sites. B_{max} values for the six individual crude homogenates were between 0.66 and 0.93 pmol/mg of protein. The average B_{max} value was 0.8 pmol/mg of protein. B_{max} for homogenate H_0 and for the microsomal fraction are 1.7 and 7.4 pmol/mg of protein, respectively. K_D for the [³H]ouabain-receptor complex were 0.6, 0.5, and 0.55 μM for the crude homogenate, for the purified homogenate H_0, and for the microsomal fraction, respectively.

Table III, which summarizes B_{max} data for [³H]en-TTX and [³H]ouabain binding indicates that, as it should be expected, B_{max} values for the two categories of ligands vary in parallel in going from the crude homogenate to the microsomal fraction. This enrichment is about twofold from the total homogenate Hg_0 to homogenate H_0 and about ninefold from the crude homogenate to microsomes.

[³H]en-TTX and [³H]ouabain binding to human muscle with MyD. Pathological samples were obtained from six patients with clinical features, electromyography and histoenzymological analyses consistent with typical MyD (Table I).

The maximum binding capacity for [³H]en-TTX and [³H]ouabain to crude homogenates of these pathological muscles were very variable from one patient to another. The reason for this high variability is that B_{max} values are expressed per milligram of protein. The

TABLE III
Comparative B_{max} Values of TTX and Ouabain on the Three Fractions of Increasing Purity (Crude Homogenate, Purified Homogenate and Microsomes)

	Crude homogenate* (Hg_0)	Homogenate (H_0)	Microsomes (P_3)
Human normal muscle			
B_{max} TTX, *fmol/mg protein*	28±4	60	230
B_{max} ouabain, *pmol/mg protein*	0.80±0.14	1.7	7.4
Human MyD muscle			
B_{max} TTX, *fmol/mg protein*	/	77	240
B_{max} ouabain, *pmol/mg protein*	/	0.27	2.4
Human nerve-impaired muscle			
B_{max} TTX, *fmol/mg protein*	34±8	93	395
B_{max} ouabain, *pmol/mg protein*	2.3±1.0	3.3	13.4

* Data for crude homogenates are averages from eight experiments in normal muscles and from twice six experiments in nerve-impaired muscles. Data for homogenates H_0 and microsomes are obtained from pooled muscle samples (Results).

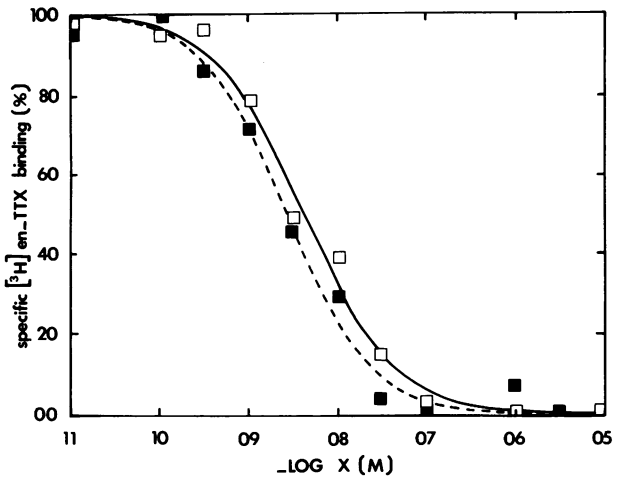

FIGURE 3 Unlabeled TTX and STX antagonism of specific [^{3}H]en-TTX binding to crude homogenate from normal human muscle at 4°C. Half-maximum effects were obtained with TTX (□) and STX (■) at 4.1 and 2.7 nM, respectively. Concentrations of [^{3}H]en-TTX were 1.6 nM for competition by TTX and 1.1 nM for competition by STX. Each point was made in duplicate by filtration of 0.4 ml of incubation medium containing 0.5 mg of protein/ml of homogenate on GF/B filters.

different samples of myotonic muscles have undergone a variable loss of proteins, mainly of contractile proteins. Therefore, it is of limited significance to work directly on crude homogenates. It is necessary to introduce a partial purification of the external membrane system before performing binding assays.

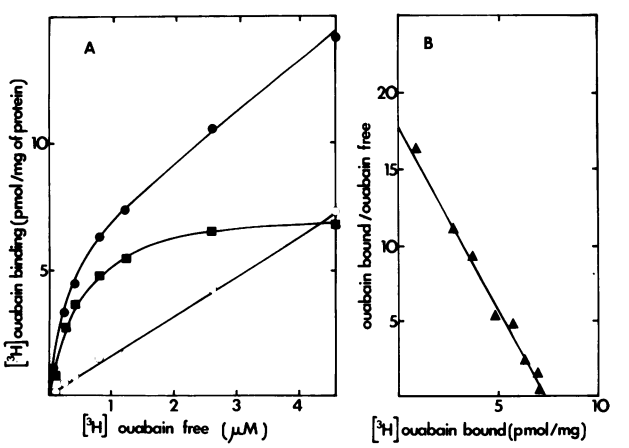

FIGURE 4 Binding of [^{3}H]ouabain to microsomes from normal human skeletal muscle at 25°C. (A) Total [^{3}H]ouabain (●) and nonspecific binding (○) were measured in parallel in the absence or in the presence of 1 mM ouabain. Specific binding (■) was obtained from total and nonspecific binding. K_D and B_{max} values of 0.51 μM and 7.2 pmol/mg of protein were obtained, respectively. (B) A Scatchard plot of these binding data gave K_D and B_{max} values of 0.55 μM and 7.4 pmol/mg of protein, respectively.

Linear Scatchard plots for both [^{3}H]en-TTX and [^{3}H]ouabain binding were obtained for the purified homogenates H_0 partially free of contractile proteins and for the microsomal fraction.

B_{max} values for [^{3}H]en-TTX binding were 77 and 240 fmol/mg of protein for homogenate H_0 and for the microsomal fraction, respectively. These values are very similar to those found for normal human muscle (Table III).

B_{max} values for [^{3}H]ouabain binding were 0.27 pmol/mg of protein in homogenate H_0 and 2.4 pmol/mg of protein in the microsomal fraction. The number of ouabain binding sites was therefore decreased by a factor of 3-6 as compared with normal human muscle (Table III).

K_D for the [^{3}H]en-TTX-receptor and for the [^{3}H]ouabain receptor were found to be 0.9 nM and 0.8 μM, respectively, both with homogenate H_0 and with the microsomal fraction.

[^{3}H]en-TTX and[^{3}H]ouabain binding to muscles of patients with lower motor neuron impairment. [^{3}H]en-TTX binding studies were first carried out with crude homogenates prepared from individual muscle samples taken from six patients with motor neuron disease and from six patients with peripheral motor nerve disease (Table II). B_{max} values were between 29 and 44 fmol/mg of protein (average B_{max} = 34 fmol/mg of protein) for patients with motor neuron disease and between 26 and 41 fmol/mg of protein for patients with peripheral motor nerve disease (average B_{max} = 34 fmol/mg of protein).

Maximal binding capacities for [^{3}H]ouabain were between 2.1 and 2.3 pmol/mg protein (average B_{max} = 2.2 pmol/mg of protein) for motor neuron disease and between 1.6 and 3.2 pmol/mg of protein (average B_{max} = 2.4 pmol/mg of protein) for peripheral motor nerve disease. Considering the relative homogeneity of the results on the different pathological samples, the 12 samples from patients with lower motor nerve impairment were pooled to prepare the H_0 homogenate and the microsomal fraction.

Linear Scatchard plots both for [^{3}H]en-TTX and [^{3}H]ouabain binding were obtained on both homogenate H_0 and on microsomes. B_{max} values for TTX receptors were 93 fmol/mg of protein using H_0 and 393 fmol/mg of protein using microsomes. Values for the ouabain receptor were 3.3 pmol/mg of protein (H_0) and 13.4 pmol/mg of protein (microsomes).

K_D for the [^{3}H]en-TTX-receptor and for the [^{3}H]ouabain receptor complex were 0.8 nM and 0.4 μM, respectively. These values are nearly identical to those found in normal and myotonic muscle.

The yield of membrane purification calculated from the enrichment in [^{3}H]en-TTX and [^{3}H]ouabain receptors when going from the crude homogenate to mi-

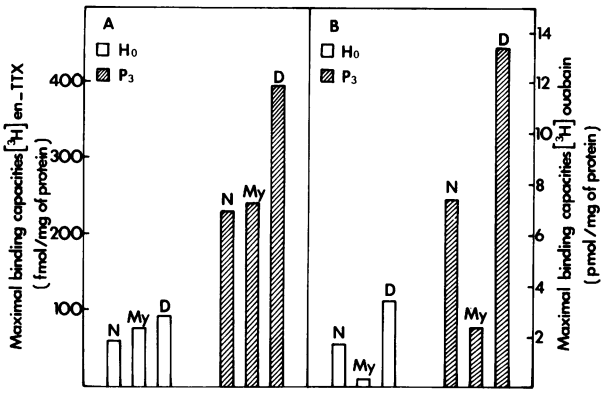

FIGURE 5 Comparison between TTX (A) and ouabain (B) binding capacities in normal muscle (N), muscle from patients with myotonic muscular dystrophy (My) and nerve-impaired human muscle (D). Purified homogenate H_0 and microsomes P_3 were prepared from a pool of biopsies in normal subjects, biopsies from patients with MyD and biopsies from patients with lower motor nerve impairment.

crosomes is the same for normal and pathological muscle. Finally, the comparison of binding capacities for [^{3}H]en-TTX and [^{3}H]ouabain in normal, myotonic and nerve-impaired human muscle is shown in Fig. 5.

DISCUSSION

The first series of experiments presented in this paper relate to the properties of the receptors for TTX and ouabain in normal human muscle. The concentration of ouabain receptors in microsomes (7.4 pmol/mg of proteins) is 32 times higher than the concentration of TTX receptors (230 fmol/mg of protein). K_D found for the ouabain-receptor complex, 0.55 μM, are similar to those found for rat muscle 0.2-0.22 μM (22, 23). K_D found for the [^{3}H]en-TTX-receptor complex, 0.5 nM, are comparable to those obtained for cardiac cells (19), rat synaptosomes (24) and rat muscle (25); in the latter two systems [^{3}H]STX was used under the same conditions.

The [^{3}H]en-TTX binding was selectively antagonized by unlabeled TTX and STX. Affinities found for TTX (K_D = 1.0 nM) and for STX (K_D = 0.85 nM) are similar to those found in membranes of cardiac cells (19), synaptosomes (24), and rat muscle (25).

The molecular basis of muscular dystrophies and of other diseases of the nerve-muscle unit is still not clearly established with the exception in the case of myasthenia gravis that is believed to be due to the formation of antiacetylcholine receptor antibodies (26).

MyD. Electrophysiological investigations of MyD have shown that high frequency repetitive discharges were induced by needle movement, percussion or voluntary activity. Thus, myotonia is best defined as a sustained contraction of muscle fibers caused by repetitive membrane depolarization, which occurs in the absence of nerve influence since it persists after curarization. At least two mechanisms have been proposed to explain two different types of myotonic disorders. In hyperkaliemic familial periodic paralysis and in the closely related disease paramyotonia congenita, external membranes of muscle fibers probably depolarize as a result of an increased permeability to Na^+ (27). Other studies performed with hereditary myotonic goats have led to the suggestion by extrapolation that human myotonia congenita results from a reduction of membrane permeability to chloride. In myotonia congenita, the resting membrane potential is not changed and a normal size depolarization is still required to initiate action potentials (28). However, a small variation of Na^+ conductance could in that case exert a stronger than normal depolarizing action since it could only be balanced by potassium channels and not, as in normal membranes, by the combined effects of potassium and chloride channels.

Anomalies of chloride channels have not yet been detected in MyD (29). This disease is known to be a systemic genetic disorder inherited as an autosomal dominant trait. Electrophysiological studies have shown that the resting membrane potential is reduced (28, 30–33) and that the membrane resistance is slightly increased (34). The overshoot of the action potential is not significantly affected (32) or only slightly decreased (33) and, like in normal muscle, the action potential is completely abolished after a 3-min exposure to 1 μM TTX (7, 33). These results may suggest that the sodium pump, which controls in part the resting potential, is altered in MyD while the sodium conductance that is inhibited by TTX is normal, or only very slightly modified (33).

A different approach taken to determine the molecular defects in MyD consists in analyzing the properties of erythrocyte membranes of patients with MyD. Roses et al. (35, 36) showed that phosphorylation of band III by an endogenous protein kinase was deficient in erythrocyte ghosts from patients with MyD. Band III contains several proteins including (Na^+, K^+)ATPase. Erythrocyte membranes from patients with myotonia congenita or Duchenne muscular dystrophy had properties similar to normal membranes (37).

A change in the stoichiometry of Na^+ and K^+ transport catalyzed by the (Na^+, K^+)ATPase has also been shown in erythrocyte membranes of patients with MyD. A "normal" (Na^+, K^+)ATPase catalyzes the extrusion of three sodium ions and the coupled influx of two potassium ions (38). The pathological (Na^+, K^+)ATPase has been reported to have a stoichiometry

of transport on only two sodium for two potassium ions (39). An alteration of calcium transport was also reported for erythrocytes of patients with MyD (40).

Data presented in this paper have shown that voltage-sensitive Na^+ channels are apparently normal in MyD. Their number expressed per milligram of protein (Table III) as well as the stability (K_D) of their association with [^{3}H]en-TTX are nearly identical to normal muscle. The situation is completely different for ouabain binding. Although the equilibrium dissociation constant of the [^{3}H]ouabain-receptor complex is the same in normal and myotonic muscle, there is a three- to sixfold reduction of ouabain binding sites in muscles with MyD. This large difference in the number of sodium pumps might be the origin of the reduced resting membrane potential (35, 39) and of the slightly increased membrane resistance (34).

Lower motor neuron impairment. The loss of motor innervation induces marked morphological changes in muscle fibers and drastic alterations of the external membrane system. These effects are attributed to the withdrawal of the trophic influence normally exerted by motor neurons on muscle fibers (41). Two classical examples of these alterations are the increase in number of acetylcholine receptors after denervation (42, 43) and the appearance in animal muscle fibers of TTX-resistant action potentials (44, 45). 10–15 d after total experimental denervation of rat muscle, Ritchie et al. (46) and Barchi et al. (25) using [^{3}H]STX to titrate sodium channels, have observed a decrease of the order of 33 to 43% in the number of saxitoxin binding sites. The affinity of saxitoxin for its receptor was the same in innervated and denervated muscle (25, 46).

In contrast with the observations made after experimental denervation of rat muscle, Gruener et al. (7, 33) reported that TTX-resistant action potentials are not observed in pathologically nerve-impaired human muscle. This absence of TTX resistance suggests an incomplete loss of nerve control in pathological denervations and, of course, unchanged properties for the fast sodium conductance.

Data obtained in this paper (Table III) show in contrast with observations made on rat muscle (25, 46), that there is an increase by a factor of 2 in the number of TTX binding sites on nerve-impaired human muscle as compared with normal human muscle. This change in number of sites is not accompanied by a change in the affinity of sodium channels for [^{3}H]en-TTX.

The number of ouabain binding sites in nerve-impaired human muscle is twofold higher than in normal human muscle. The affinity of ouabain for its receptor is the same in normal and pathological muscles. A similar increase in the number of ouabain binding sites was observed by Festoff et al. (23) after denervation of rat muscle.

Electrophysiological recordings in human nerve-impaired muscle have shown fibrillation potentials. These potentials reflect a defective ionic balance in response to denervation of the muscle membrane itself since they are unaffected by curarization. In addition, a severe loss of membrane excitability linked to a reduced input membrane resistance has been observed by Gruener (7, 33) on muscles with motor neuron disease. These different electrophysiological properties of the pathological muscle may be due to the increased number of Na^+ channels.

One of the problems with both denervated muscle and myotonic muscle is that there is an architectural disruption which is secondary to the illness rather than being primary. Experiments carried out in this paper describe binding data obtained at different stages of membrane purification and make it unlikely that the variations in number of ouabain and TTX binding sites are simply the result of membrane disruption. However, since both normal human muscle and myotonic muscle can now be grown in culture (47) and since electrophysiological abnormalities demonstrated in myotonic myotubes suggest similarities of the intact fiber and of the tissue culture system (47), it would now be of a great interest to apply the findings reported in this paper to cultures of normal and myotonic muscle.

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