The correlation between electrical and mechanical after-activity was studied in resealed fiber segments from patients with recessive generalized myotonia (Becker) and in intact fibers from normal muscles which were bathed in 9-anthracene carboxylic acid. The tests were performed in vitro on small bundles of 100 fibers or less. Electromyographic activity and contractile force were measured simultaneously. The relaxation of rested-state twitches and tetani was slowed and accompanied by after-activity in both types of preparations. Often random activity was recorded. In all cases, the contractile force was highly correlated with the electromyographic signs of myotonia. These observations support the hypothesis that electrical after-activity is fully responsible for the slowed relaxation in recessive generalized myotonia.
Key words: in vitro measurements • electromyograms • force • recessive generalized myotonia • 9-anthracene carboxylic acid

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THE CORRELATION BETWEEN ELECTRICAL AFTER-ACTIVITY AND SLOWED RELAXATION IN MYOTONIA

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Involuntary electrical after-activity following muscle activation has been suggested to be responsible for the muscle stiffness in myotonia.[3,7] Several investigators have questioned this idea; their doubts were based on the disproportionality between the intensities of the electrical activity and the stiffness observed in vivo within the various myotonic disorders.[9,10,13,14] It is difficult to be certain that the electrical activity recorded in vivo from one muscle region is representative of the total involuntary activity producing the observable stiffness. We hoped to eliminate this uncertainty by simultaneously recording, in vitro, electrical activity and force from very thin bundles of myotonic muscle.

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PATIENTS, MATERIALS, AND METHODS

Four male patients with recessive generalized myotonia were investigated. For each of the patients the following was true: (1) the first signs of myotonia became evident when he was in his late teenage years; (2) compared with the legs, the upper limb and shoulder girdle muscles were poorly developed and affected by transient weakness (occurring regularly after rest; improving with exercise); (3) severe myotonia was recorded electromyographically from all muscles and percussion myotonia was regularly elicitable; (4) no other organs appeared to be involved in the disease process (e.g., no cataracts were detected); and (5) no myotonic symptoms were known to exist in any other family members. The patients were chronologically numbered for easier cross-reference between our papers.

From these myotonic patients muscle specimens with lengths ≥2.5 cm were obtained from the motor point region of the biceps brachii under local anesthesia. Under general anesthesia, specimens of the external intercostal muscle were obtained from four control patients with no known neuromuscular disease who had to undergo thoracic surgery. All procedures were in accordance with the Ethics Committee of the Technical University of Munich and the Helsinki convention.

The excised muscle specimens were transported to the laboratory in gassed (95% O_2, 5% CO_2) Bretag solution at room temperature. The

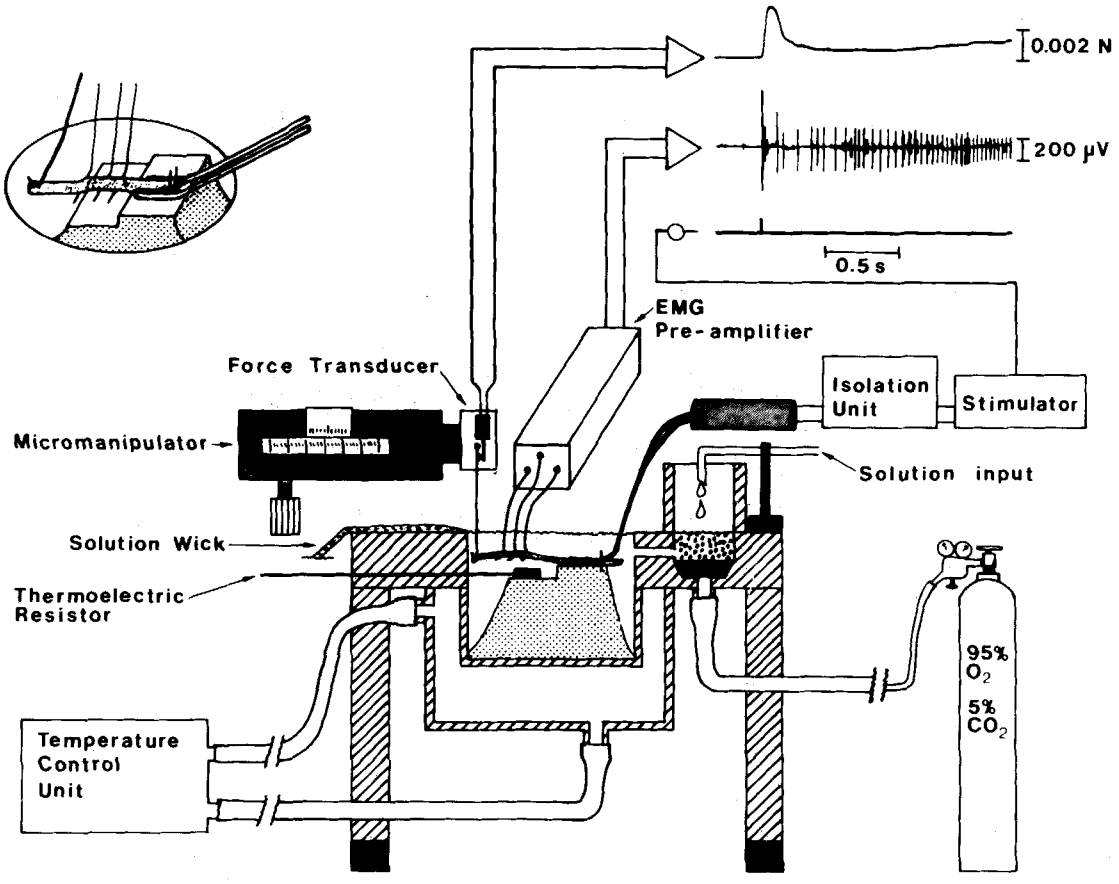

FIGURE 1. The experimental set-up. The inset in the upper left-hand corner shows the muscle bundle suspended over the EMG electrodes and connected to the force transducer. Three signals were simultaneously recorded: force, the electromyographic activity, and the stimulator output (e.g., the signals shown were those recorded from fiber segments prepared from a patient with recessive generalized myotonia, RGMy4).

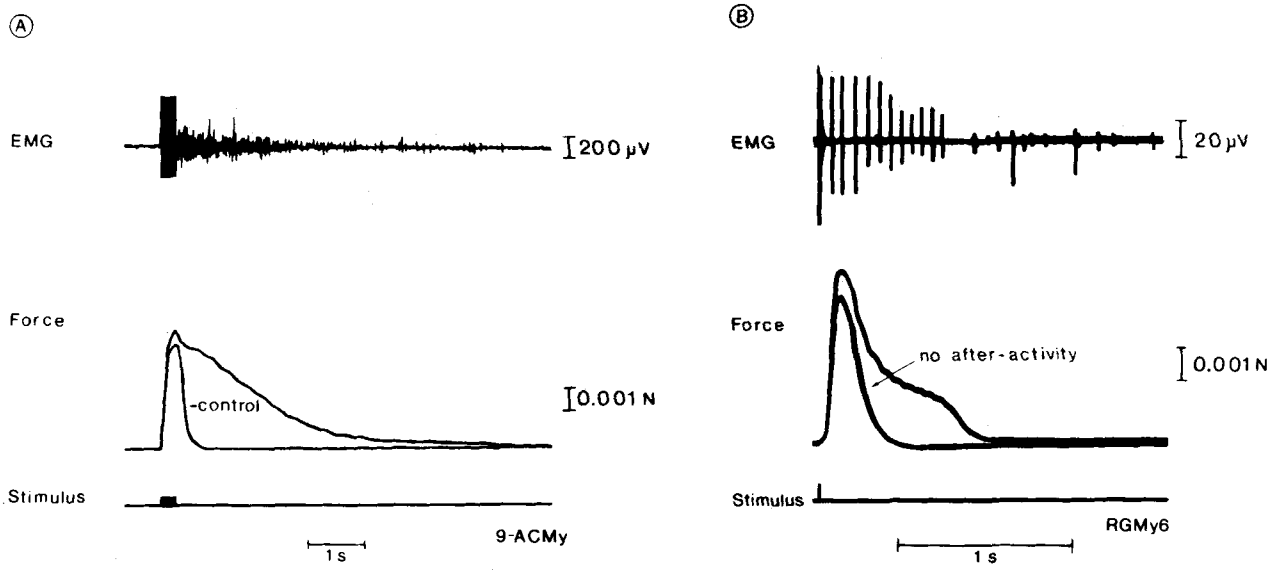

FIGURE 2. Myotonic activity recorded from intact fibers bathed in 9-AC and from fiber segments obtained from a patient with recessive generalized myotonia (RGMy6). (A) Drug-induced myotonia: the response of intact fibers before and after exposure to 0.05 m*M* 9-AC (stimulated at 30 Hz). Only the electromyographic activity following drug exposure is shown. (B) A rested-state twitch response of the fiber segments showing spontaneous activity and a subsequent response (10 seconds later) which elicited no after-activity (not shown).

specimens were dissected into thin (2–8 fibers thick) bundles of ≤100 of either intact fibers or long fiber segments (≥2.5 cm). The intact fibers from normal muscles were made myotonic by bathing them in a solution containing by 9-anthracene carboxylic acid (9-AC; 0.01 or 0.05 m*M*; Aldrich Chemicals Inc., Milwaukee, WI).[2,4,6] The fiber segments prepared from the muscles from the patients with recessive generalized myotonia were allowed to reseal and recover for at least 2 hours after dissection. Prior to electrical stimulation, the resting membrane potentials of these fiber segments were measured by means of glass microelectrodes filled with 3 *M* KCl which had a resistence of 3.5 M ohm.

The force and electromyographic (EMG) activity of a bundle was recorded within an experimental chamber which was continuously perfused with solution under continuous gassing with 95% O_2 and 5% CO_2. The EMG activity was monitored via 2 silver wires which were placed 6 mm apart (see Fig. 1). A third silver ground wire was nearby. The muscle bundle was suspended over these wire electrodes while securing one end to a force transducer (Akers, Horten, Norway). In order to increase the EMG signal-to-noise ratio, the bathing solution in the chamber was lowered momentarily before and after stimulation. The fibers were stimulated via two additional silver wires running parallel along the length of the bundle (approximately for 0.6 cm). Supramaximal current pulses of 0.2 msec duration were either delivered individually or in trains of 300 msec duration at various intervals.

The bathing solution (Bretag) contained 107.7 m*M* NaCl, 3.5 m*M* KCl, 1.58 m*M* $CaCl_2$, 0.7 m*M* $MgSO_4$, 1.7 m*M* NaH_2PO_4, 26.2 m*M* $NaHCO_3$, 9.64 sodium gluconate, 5.5 m*M* glucose, and 7.6 m*M* sucrose. The pH was adjusted to 7.4 by bubbling this solution with a mixture of 95% O_2 and 5% CO_2, and all experiments were conducted at 37°C.

RESULTS

The resealed fiber segments, obtained from the myotonia patients, recovered to have normal resting membrane potentials of −82.1 ± 6.7 mV (n = 77; $\bar{X}$ ± SD). These fiber segments displayed myotonic patterns typically described in vivo and for excised fibers intact from tendon to tendon.[14,15] These patterns included (1) myotonic runs lasting 1–2 seconds with modulation of frequency and amplitude which were induced by electrode impalement, and (2) electrical after-activity and associated slowed relaxation following either twitch or tetanic stimulation. Because of the thin diameter of the bundles which were investigated here, it was possible to record the electrical activity of every active fiber. Furthermore, each time we observed spontaneous electrical potentials, we detected associated increases in contractile force. Conversely, spontaneous force development and slowed relaxation were accompanied by electrical activity in all preparations (n = 15). On the other hand, not in every individual response (e.g., the initial responses as the preparation was being adjusted within the recording chamber) was a correlation between the electrical activity and force observed. However, in all such cases the dissociation could be compensated for. These dissociated re-

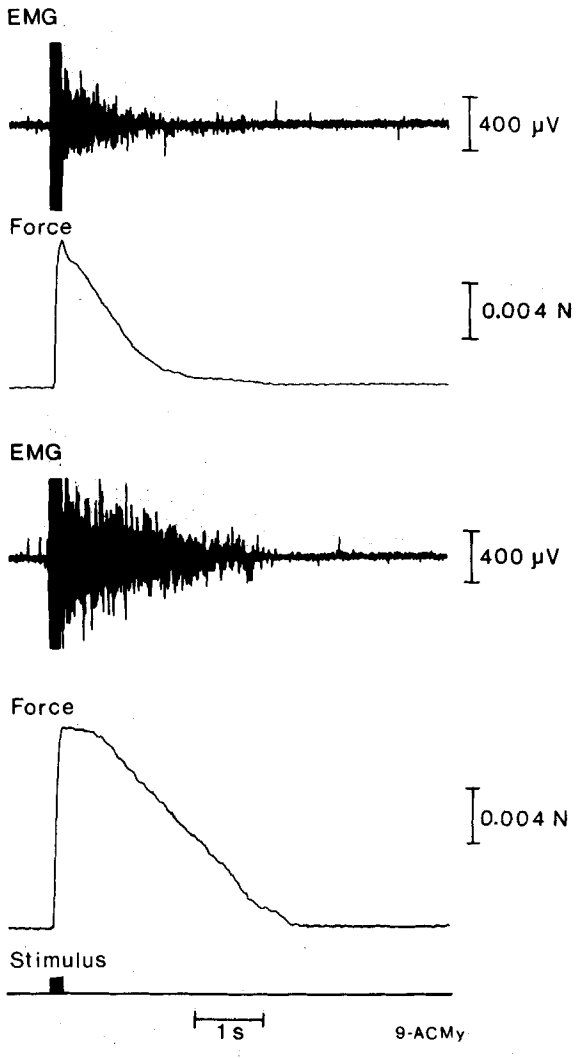

FIGURE 3. High correlation between duration of electrical after-activity and the decay of force. Both responses were recorded from the intact fibers (bathed in 0.05 m*M* 9-AC). The fibers were rested and supramaximally stimulated (30 Hz) for 300 ms.

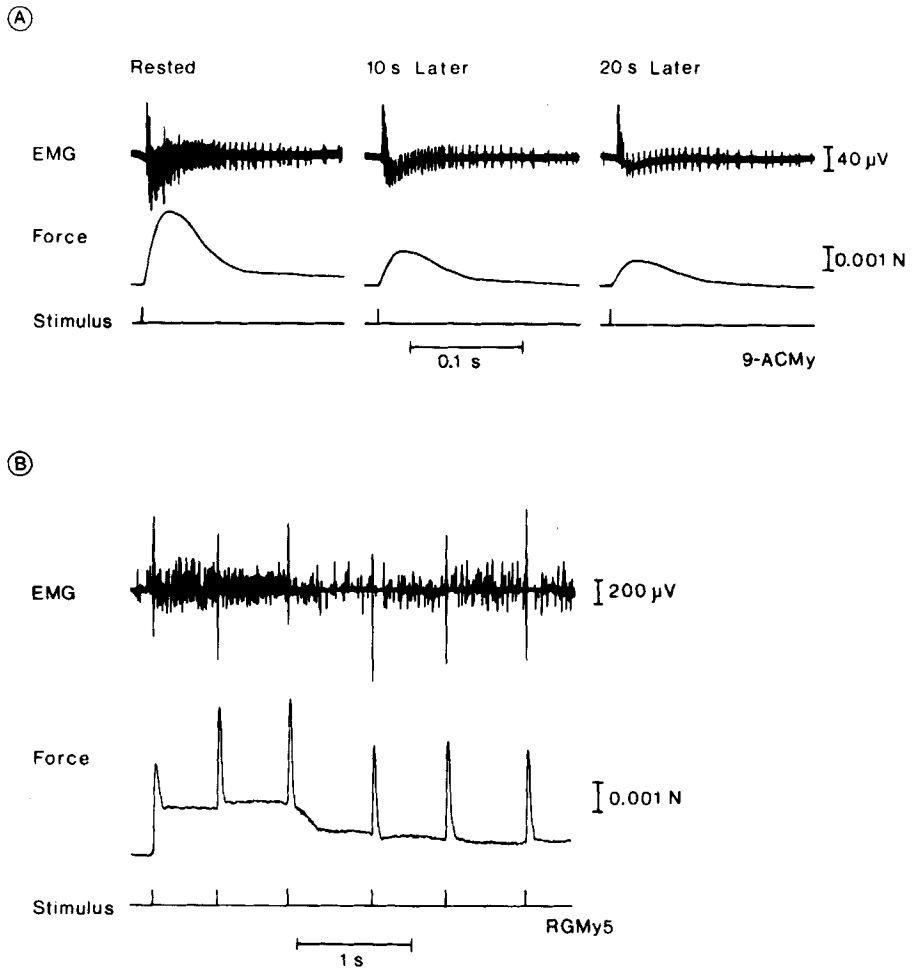

FIGURE 4. Decreased electrical after-activity following successive stimulation. (A) Three successive twitch responses of intact muscle fibers bathed in 0.05 m*M* 9-AC. (B) Six successive twitches of fiber segments from a patient with recessive generalized myotonia (RGMy5). The after-activity subsequently decreased.

sponses resulted from the following experimental errors: (1) excessive friction between the preparation and the recording system caused by the reduction in the solution level, (2) overstretch of a preparation which caused the rate of relaxation to be decreased as described by Rack and Westbury,[12] and (3) excessive stimulation of a preparation (over a period of several hours) which caused the viability of the preparation to decrease and/or the depletion of ATP (this behavior was similar in both the intact fibers [treated and untreated] and the fiber segments).

In both the resealed fiber segments from the myotonia patients and the normal intact fibers bathed in 9-AC, the relaxation of rested-state twitches and tetani was slow and accompanied by electrical activity (Fig. 2). The drug-induced after-activity often lasted for several seconds. The intensity and duration of the after-activity was dependent on the concentration of 9-AC that was used: the higher concentrations induced a more pronounced slowed relaxation and an increased amount of associated electrical after-activity. Moreover, there existed a consistent linear relationship between the duration of the electrical after-activity and the return of force to resting levels (Fig. 3). This behavior was identical to that previously reported for recessive generalized myotonia.[15] In subsequent contractions the relaxation became faster and electrical after-activity decreased. This warm up phenomenon was evident in bundles from the patients with recessive generalized myotonia and in the drug-induced myotonia (Fig. 4). The amount of after-activity decreased with successive twitches. The peak amplitudes of the initial twitches were augmented

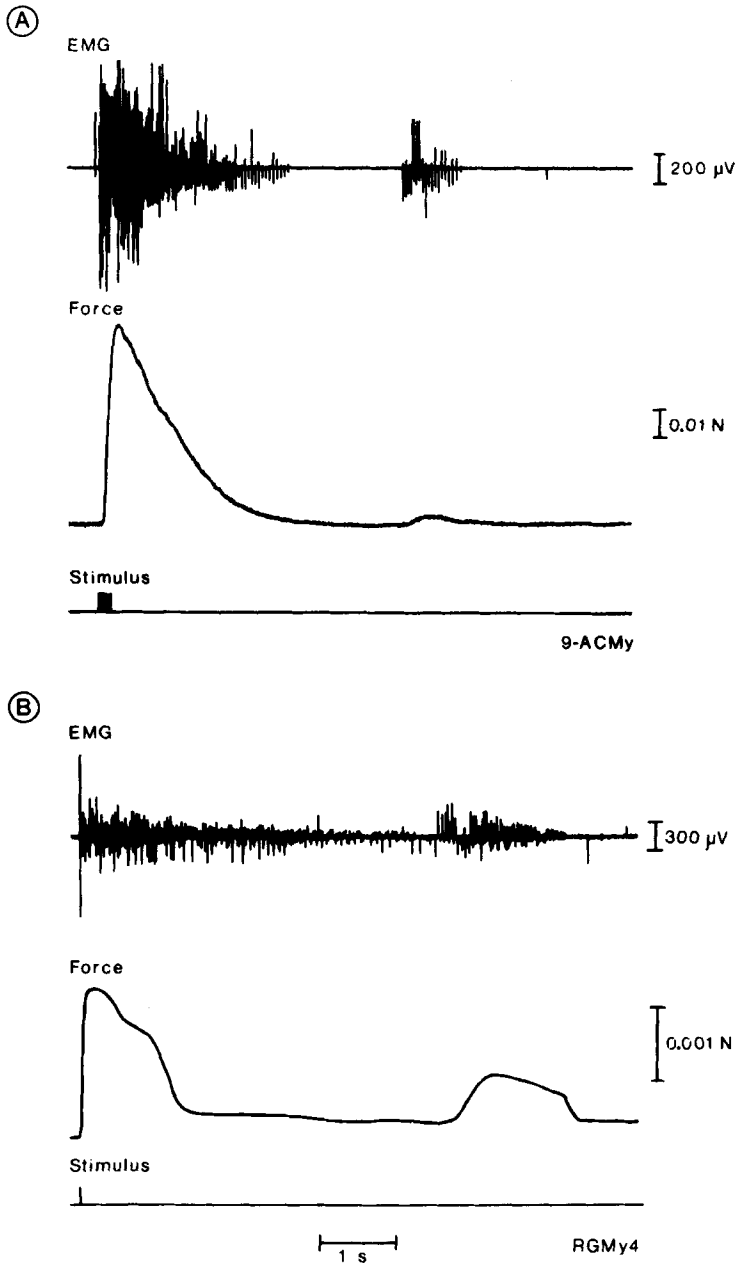

FIGURE 5. Random bursts of myotonic activity. Intense myotonic activity was observed following stimulation in both (A) the drug-induced myotonia (0.05 m*M* 9-AC, stimulation 30Hz) and (B) the fiber segments obtained from a myotonic patient (RGMy4, a twitch response). The subsequent spontaneous bursts of electrical activity were associated with increases in force.

by the after-activity. The rapid onset of high-frequency myotonic activity produced mechanical responses which were similar to those described for high-frequency (200 Hz) multiple-pulse stimulations of mammalian muscle.[11] In Fig. 4B, the twitches appeared to be superimposed on a tetaniclike response produced by a fairly constant level (for 1 second) of spontaneous electrical activity.

Random spontaneous electrical activity was recorded from both types of preparations which was well correlated to force. Examples of spontaneous "myotonic runs" are shown in Figs. 5, 6, and 7B. From several preparations of either intact fibers bathed in 9-AC or fiber segments from the myotonic patients it was possible to record long-lasting low-frequency spontaneous twitching of one or more fibers (Fig. 7). Note that in every record de-

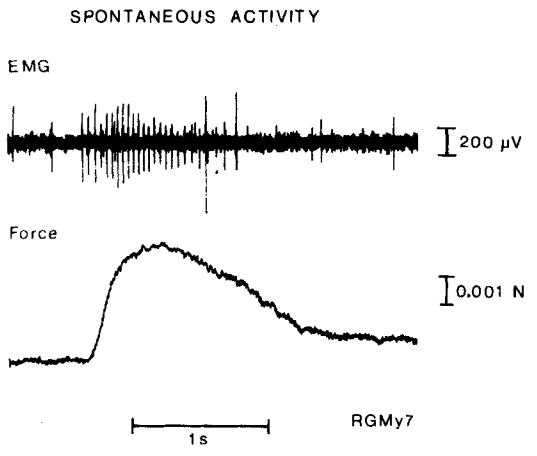

FIGURE 6. A random "myotonic run." This activity was recorded from the fiber segments obtained from a patient with recessive generalized myotonia (RGMy7). There was a good correlation between the duration of the electrical and mechanical activity.

tectable contractile force was produced by each electrical potential.

DISCUSSION

The high correlation shown here between the electrical and mechanical activity confirms the hypothesis that stiffness in certain myotonic conditions is due solely to spontaneous electrical events that activate normal contractile elements.[3,7] This was true for the resealed fiber segments prepared from muscle specimens obtained from patients with recessive generalized myotonia and for intact fibers from normal subjects which were made myotonic by blockage of Cl^- channels. Drug-induced myotonia using 9-AC has been suggested to be a good model of Thomsen's myotonia congenita.[2,4,6] Thus, it may be true that in both recessive generalized myotonia and myotonia congenita a high correlation exists between electrical and mechanical after-activity. However, it should be noted that in paramyotonia congenita it has been reported that such a correlation does not exist.[13] In this myotonic disorder, an additional defect of the contractile apparatus has been suggested.[13]

Intact muscle fibers bathed in 9-AC showed electrical and contractile properties of fibers obtained from patients with recessive generalized myotonia.[15] 9-AC has been reported to affect only the chloride conductance of a muscle,[6] which also has been reported as one of the major defects in hereditary myotonia.[1] The similar in vitro behavior of the drug-induced myotonia and the behavior of the fiber segments from the patients with recessive generalized myotonia adds support to

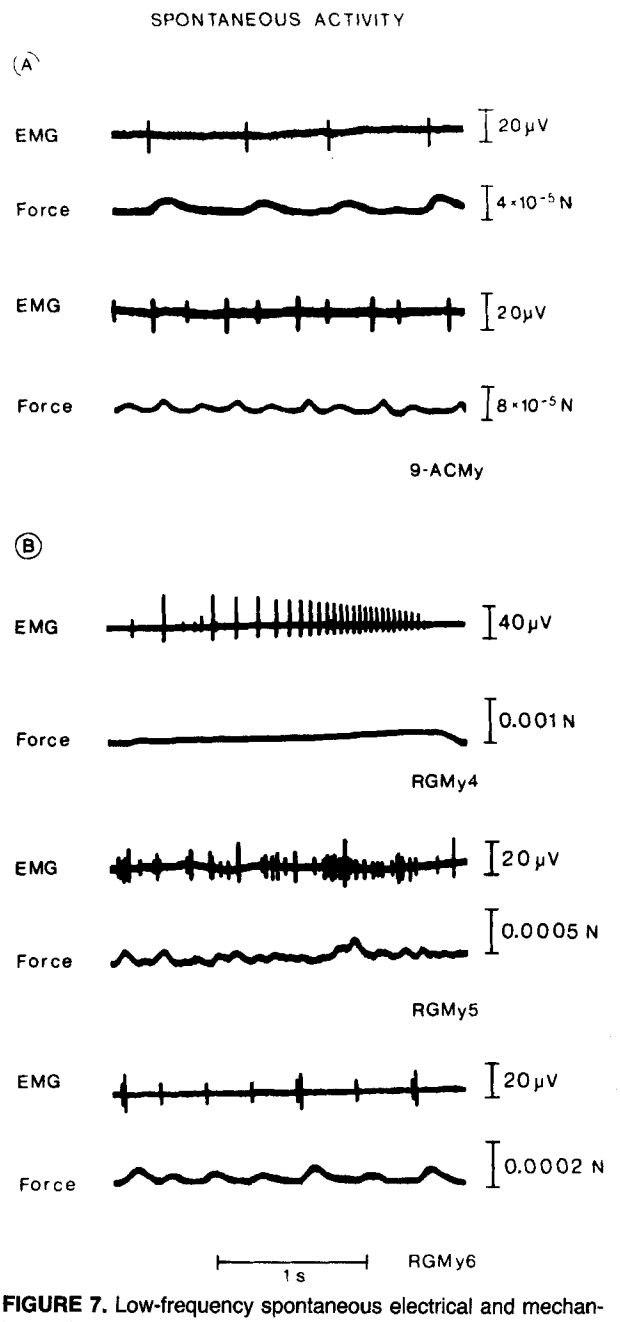

FIGURE 7. Low-frequency spontaneous electrical and mechanical activity. Each electrical spike produced an increase in force. (A) Two records of such activity in drug-induced myotonia (0.05 m*M* 9-AC). In the upper record, only one fiber was twitching and in the lower record two different fibers were active. (B) Three records of spontaneous myotonic activity recorded from fiber segments obtained from three different myotonia patients (RGMy4, 5, 6). In the middle record at least three different fibers were active. In the lower record two different fibers were twitching.

the theory that this is a good model for the clinical condition. In addition, it may also support the suggestion that a reduced chloride conductance may be one of the primary causes of the myotonic

activity in certain patients with recessive generalized myotonia.[15]

Long fiber segments obtained from normal muscles repolarize and possess electrical and mechanical properties of intact fibers.[5,8] Likewise, fiber segments obtained from patients with recessive generalized myotonia displayed myotonic activity identical to that previously observed in vivo and in vitro.[14,15] This finding emphasizes the usefulness of fiber segments for the investigation of the pathophysiology of human myotonic disorders. In summary, our observations support the hypothesis that in recessive generalized myotonia the contractile apparatus and uptake of Ca^{2+} are normal and the electrical after-activity is responsible for the slowed relaxation.

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