Intrinsic Properties and Reflex Compensation in Reinnervated Triceps Surae Muscles of the Cat: Effect of Activation Level

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Huyghues-Despointes, Clotilde M.J.I., Timothy C. Cope, and T. Richard Nichols. Intrinsic properties and reflex compensation in reinnervated triceps surae muscles of the cat: effect of activation level. J Neurophysiol 90: 1537–1546, 2003. First published May 7, 2003; 10.1152/jn.00718.2002. The manner in which activation levels influence intrinsic muscular properties and contributions of the stretch reflex were studied in homogeneous soleus (SOL) and heterogeneous gastrocnemius (G) muscles in the decerebrate cat. Intrinsic mechanical properties were represented by the initial stiffness of the muscle, measured prior to reflex action, and by the tendency of the muscle to yield during stretch in the absence of the stretch reflex. Stiffness regulation by the stretch reflex was evaluated by measuring the extent to which reflex action reduces yielding and the extent to which stiffness depends on reflex action. Intrinsic mechanical properties were measured on muscles deprived of effective autogenic reflexes using the method of muscular reinnervation. Reinnervated muscles were recruited to force levels comparable to those achieved during natural locomotion. As force declined during crossed-extension reflexes in reinnervated and intact muscles, initial stiffness declined according to similar convex trajectories. The data did not support the hypothesis that, for a given force level, initial stiffness is greatest in populations of predominantly type I motor units. Incremental stiffness (Δl/Δl) of both G and SOL increased in the presence of the stretch reflex. Yielding of SOL (ratio of incremental to initial stiffness) substantially decreased in the presence of the stretch reflex over the full range of forces. In reflexive G, yielding significantly decreased for low to intermediate forces, whereas at higher forces, yielding was similar irrespective of the presence or absence of the stretch reflex. The stretch reflex regulates stiffness in both homogeneous and heterogeneous muscles.

INTRODUCTION

Pathways underlying stretch-evoked reflexes have been shown to contribute importantly to the mechanical properties of muscles in both reduced animal preparations (Cope et al. 1994; Cordo and Rymer 1982; Hoffer and Andreassen 1981; Houk et al. 1970; Liddell and Sherrington 1924; Nichols and Houk 1976) and in intact animals and human subjects (Allum and Mauritz 1984; Carter et al. 1990; Henneman and Mendell 1981). These observations have led to the suggestions that proprioceptive pathways contribute to the regulation of anti-gravity support during standing (Nichols 2002; Sherrington 1906) and locomotion (Pearson et al. 1999; Stein et al. 2000) and regulate the characteristics of the mechanical interface between body and environment (Feldman 1986; McMahon and Greene 1979) in conjunction with the intrinsic mechanical properties of the musculoskeletal system (Granit 1958; Houk and Rymer 1981; Joyce et al. 1969; Matthews 1959; Rack 1970).

A specific proposal concerning a physiological role of short-latency feedback is that pathways underlying the stretch reflex regulate the mechanical properties of muscle and contribute to net muscular stiffness after a brief delay (Houk et al. 1981; Nichols 1992, 1994; Nichols and Houk 1973; Rack 1970). When a muscle contracts isometrically and is then subjected to a constant velocity stretch, the initial response is determined solely by intrinsic mechanical properties. In the absence of reflex action, the muscle then yields. That is, the stiffness of the muscle declines abruptly beyond a short range. In the presence of the stretch reflex, an increased firing rate modulation and a recruitment of fresh motor units maintain muscular stiffness beyond the short-range. The result is that the stretch reflex compensates for muscle yielding with a consequent increase in linearity of the response, an increase in incremental stiffness of the muscle, and a reduction of the dependence of incremental stiffness on background force (Hoffer and Andreassen 1981; Nichols 1974, 1985; Nichols and Steeves 1986). In the aforementioned studies, the intrinsic mechanical properties of muscles were estimated using either dorsal rhizotomy or direct electrical stimulation of ventral roots or muscle nerve. Further evidence for reflex compensation was obtained in experiments on human subjects in which intrinsic properties were determined using electrical stimulation of the muscle (Carter et al. 1990; Sinkjer 1997). A more recent test of the stiffness hypothesis was performed by disrupting localized proprioceptive feedback to the triceps surae muscles in cats (Abelew et al. 2000). During downhill walking, motor deficits observed during the stance phase of stepping were consistent with a reduction in ankle joint stiffness, suggesting a reduction in the stiffness of the triceps surae muscles during active lengthening.

As compelling as these arguments are about the roles of proprioceptive pathways and intrinsic properties in motor coordination, there is much to be discovered about the manner in which these mechanisms contribute to muscular mechanics over wide ranges of forces and behaviors. Most skeletal muscles are composed of more than one fiber type, and the systematic changes in motor-unit composition that accompany orderly recruitment would be expected to influence the intrinsic properties of the muscle (Burke 1981; Cope and Clark 1991;
Henneman and Mendell 1981). It has been shown that initial stiffness is less in type II than in type I motor units, suggesting that stiffness should increase less than proportionately with increasing background force (Petit et al. 1990). In addition, the extent of yielding has been thought to be less in type II than in type I muscle fibers (Malamud et al. 1996; Stienen et al. 1992), suggesting that reflex compensation might be less important at higher forces in muscles of mixed fiber type composition. Although reflex compensation for yielding might be less important under these conditions, the stretch reflex would still be expected to reduce the dependence of stiffness on background force. Finally, it has been shown that prior movement tends to linearize the transient mechanical properties of muscle (Huyghues-Despointes 1998; Kirsch et al. 1994) and reduce the initial burst of muscle spindle receptors (Gregory et al. 1990; Huyghues-Despointes et al. 1997). The role of the stretch reflex in regulation of the muscle under these conditions remains to be clarified.

The studies described here were undertaken to test the stiffness hypothesis and measure initial stiffness under the first set of conditions described in the preceding text, namely, changes in the level of recruitment with consequent changes in the sizes and types of motor units. We required an experimental preparation in which heterogeneous and homogenous muscles could be recruited physiologically in the presence and absence of autogenic reflexes. The soleus muscle (SOL) can be recruited physiologically after acute deafferentation in the decerebrate cat with transected dorsal roots (Kirsch et al. 1994), but activation of the gastrocnemius muscle (G) is generally poor. We used the procedure of reinnervation that has been shown to result in successful motor reinnervation (Cope and Clark 1993; Cope et al. 1991) with permanent disruption of the stretch reflex in triceps surae muscles of the cat (Cope et al. 1994). We confirm that reinnervation successfully disrupts reflex action across a wide range of activation levels in SOL and G and therefore constitutes a useful approach for the study of the intrinsic mechanical properties of physiologically recruited muscles. Contrary to the hypothesis of Petit et al. (1990), the relationships between initial stiffness and force were similar for both SOL and G. The extent of yielding was shown to be greater for SOL than for G at lower forces, but these differences diminished at higher forces. Prior results on the linearizing action of reflexes in SOL were confirmed and extended to the homogenous G. These data have appeared in a dissertation (Huyghues-Despointes 1998) and in preliminary form (Huyghues-Despointes et al. 1997).

METHODS

Selection and housing of animals

Seven neutered adult cats (6 males and 1 female) were selected (4.00–7.25 kg, average: 5.64 kg). The animals were housed in a large colony with other cats to allow normal and unrestricted motor activities. After surgical intervention (treatment discussed in the following text), the animals were first isolated for a recovery period that varied (1–3 mo) with individual cats prior to being reintroduced to the colony. Animals were monitored daily for general health. No stress was noted as a result of the surgical procedure. Experimental protocols were approved by Emory University’s Institutional Animal Care and Use Committee.

Reinnervation

Six of seven cats were treated. One animal was kept as an untreated control. The reinnervation procedure was carried out following standard sterile surgical techniques and was similar to that used by a number of other researchers (see Collins et al. 1986; Cope and Clark 1993; Gordon and Stein 1982). Animals were initially given acepromazine and ketamine, and anesthesia was maintained with isoflurane. Through a popliteal incision, the medial gastrocnemius (MG) and lateral gastrocnemius-soleus (LG-SOL) nerves were sectioned one at a time and reattached. Special care was taken to minimize disruption of blood supply to the muscles and the associated connective tissue. The proximal and distal nerve stumps were immediately aligned and sutured together with three to four ties of nonabsorbable 22 μm (10-0) black monofilament nylon. The wound was closed, and the animal was allowed to recover with administration of analgesics (butorphanol, 0.005–0.010 mg/kg im) and prophylactic antibiotics (penicillin, 40,000 units/kg im). Once the skin sutures were removed and there was no evidence of pain, the animal was reintroduced to the colony. Within a few months, all cats could climb the fences, run, and jump with little if any sign of intervention. The cats were allowed from 9 to 23 mo of recovery from the date of surgery. This reinnervated muscle preparation permitted an evaluation of the locomotor behavior of the animals that underwent reinnervation of the triceps surae muscle group in only one hindlimb, keeping the other limb as an internal control (Abelew et al. 2000; Nichols et al. 1999a).

Terminal surgery

Each cat was deeply anesthetized with isoflurane, and core temperature was maintained at ~37 ± 1°C (mean ± SD) with a heating pad. A tracheotomy was performed, and the carotid arteries were clamped bilaterally. The left brachial vein was cannulated for administration of fluids (lactated Ringer). The patella was removed from each hindlimb, and steel rods were inserted into the shafts of the femur and tibia bilaterally, then rigidly clamped, fixing each knee at an angle of 110°. In the stereotaxic frame, the pelvis was stabilized using hip pins. The SOL and G muscles were dissected, and their tendons were freed to compare properties of reinnervated muscle in one limb to properties of untreated muscle in the other limb. A suture was inserted through the interosseous membrane around the fibula and secured as a reference marker for muscle lengths. At an ankle angle of 90°, a suture was inserted through the desired muscle tendons to approximate the corresponding initial muscle lengths (Nichols and Houk 1976). Special care was taken to avoid bruising the muscles and to maintain their blood supply. The distal tibial nerve and the sural nerve in both limbs were dissected for stimulation to generate flexion withdrawal or crossed extension reflexes. The proximal portions of the cut nerves were draped over a bipolar hook electrode for stimulation at twice threshold. Muscle temperature was maintained at 30–34°C with streams of warm mineral oil and/or a heat lamp. Each muscle was linked to a myograph in series with a linear DC motor (stiffness >200 N/mm). When possible, the muscles of both limbs were set at equal passive tension to equate the passive contributions within the same animal. Specifically, reinnervated G was set at the same background passive tension as the contralateral untreated G, which typically positioned the two muscles at similar initial lengths. An increase in fascia around the reinnervated muscles was noted during dissection. Care was taken to remove as much of this surrounding tissue as possible. The fixation devices were rigidly clamped to mechanical ground. Mechanical artifacts occurred only rarely and could always be identified. Records with substantial artifacts were rejected. One animal had an abnormal SOL: two tendons and two aponeuroses that joined one muscle belly in its untreated hindlimb. This abnormality in the untreated limb was not detected until the terminal experiment. For this cat, only results from G were reported. After muscle dissection and intercollicular decerebration (all brain matter rostral to the tran-
section was removed), gas anesthesia was terminated. At the end of the experiment, the animal was killed with an overdose of pentobarbital sodium (Nembutal, 150 mg/kg) injected intravenously or directly in the heart.

**Acute deafferentation (selective dorsal rhizotomy)**

A dorsal rhizotomy was also performed on one untreated animal. Only SOL data were reliably obtained as G could not be reflexly activated, a typical limitation of this preparation. The procedure involved performing a laminectomy and identifying then severing the dorsal L6, L7, and S1 roots. This preparation served as a control for the reinnervation procedure to detect changes in the SOL intrinsic properties that might have been caused by changes in fiber type or motor-unit reorganization after the reinnervation treatment (Gillespie et al. 1987; Gordon and Stein 1982).

**Data acquisition**

The data were acquired with a 486-based PC at 500 Hz and saved for later analysis to a hard disk. Muscle lengths were controlled with servomotors. Rotary motion was converted to linear motion using a cable and pulley system and a linear slide. Length commands to the motors were supplied to servo amplifiers (PMI Motion Technologies) at a constant rate of 2,048 Hz per channel through a dedicated D/A conversion card. A permanent copy was recorded on an eight-channel chart recorder (Gould). Forces were measured using myographs mounted on the linear slides through a universal joint to maintain alignment with the muscles. At the end of each experiment, the myograph voltage outputs were calibrated with a standard 1.0 kg mass (single point calibration of a verified linear system). Length perturbations were specified using customized software, and actual length changes were recorded through a precision potentiometer. Muscle stretches were 2 mm in amplitude over a 50-ms period, followed by a 250-ms hold phase. These parameters fell within the physiological ranges reported for the E2 phase of the cat step cycle (Goslow et al. 1973).

With the muscles secured to the myographs, the tibial or sural nerve was stimulated at twice reflex threshold (40 Hz for 20–30 s or until the background force declined to resting level) to activate the spinal circuitry that in turn activated the muscles in a physiological pattern of recruitment (Bonasera and Nichols 1994). The resultant contraction was monitored on the chart recorder, and stretches were manually triggered once the level of force had peaked. Stretches were imposed until the force had decayed to prestimulation levels. Typically, 8–22 stretches were recorded per contraction. Whenever possible, the two hindlimbs underwent the same protocol in succession to be able to compare responses obtained at the same initial force, while the reinnerved limbs underwent the same protocol in succession to be able to compare responses obtained at the same initial force, while the hindlimbs underwent the same protocol in succession to be able to compare responses obtained at the same initial force, while the reflex excitability of the animal was similar (Fig. 2). A minimum of 4 min of rest was imposed between consecutive reflex activations.

**Data processing**

The force and length data were edited off-line with customized software to remove records that contained no data or faulty length trajectories. Less than 10% of the data were discarded during editing sessions. The data were not filtered.

**Assessment of whole muscle properties**

Initial stiffness ($K_i$) was defined as the magnitude of the stiffness generated by the muscle over the initial 10% of the stretch (Fig. 1) and calculated from the slope of the force trajectory. Because of a short period of acceleration at the onset of a stretch, the measurement was made after a constant velocity of stretch was achieved. The analysis program was designed to identify and reject this acceleration period from the slope measurement. A maximum slope value spanning 10% of the stretch could always be calculated within the initial 15–20% of the 2-mm stretch, satisfying the 1% muscle length measurement criteria set forth by Joyce et al. (1969). The units of $K_i$ were expressed in N/mm. To evaluate the initial stiffness as a function of recruitment level, $K_i$ was plotted against the tension developed prior to the stretch. According to our hypothesis, the $K_i$ versus $F_i$ curves for the SOL muscle should ideally have displayed a nearly straight-line relationship throughout the range of forces the muscle develops (Houk et al. 1970) because the type of motor units was histochemically uniform. On the other hand, in the heterogeneous G muscle, the relationship between $K_i$ and $F_i$ was expected to possess greater curvature because the ratio of fast twitch to slow twitch active units increased with higher force levels. Because fast twitch units produce a lower $K_i$ per unit of $F_i$ than slow twitch units (Malamud et al. 1996; Petit et al. 1990), this should have been reflected as a decrease in muscle stiffness at higher forces compared with the lower force level stiffness measurements.

Incremental stiffness ($K_e$) was the measure of the dynamic force response obtained at the termination of the 2-mm ramp stretch (Nichols and Houk 1976) divided by the change in length (Fig. 1) and constituted a chord stiffness. The index $K_i/K_e$ was a measure of the extent to which total muscular stiffness fell below $K_i$ at the termination of the ramp stretch. This quantity was dependent on neural feedback as well as the fiber type of the active motor units and was useful to evaluate the extent of reflex disruption on the force response of a muscle (Fig. 1). A full explanation of this calculation appears in Fig. 1. A value of 1 for $K_i/K_e$ would indicate no change in the slope from the beginning of the stretch to the end of the stretch and therefore perfect stiffness regulation. Smaller values of $K_i/K_e$ indicated larger extents of yielding but if $K_e > K_i$, then the reflex enhanced the intrinsic stiffness above the level of $K_i$. Stiffness regulation was evaluated using the ratio $K_i/K_e$ as well as by the tendency of reflex action to reduce the dependence of $K_e$ on background force.
Assessment of residual reflexes after a reinnervation

The extent of reflex recovery for each reinnervated muscle was evaluated for each animal and quantified as follows. At specific time points, the force responses of a reinnervated muscle were compared with its untreated counterpart in the contralateral limb. During a crossed extension reflex activation, the force in the activated muscles of interest gradually decayed back to the passive level. Therefore a baseline was estimated by computing a least squares fit through the first and last 50 ms of data for each stretch cycle, and it was subtracted from each force record (Bonasera and Nichols 1994). The response was defined as the absolute force generated at any point minus the baseline force estimation at that point. Two force points were considered in the analysis: the “dynamic response” measured at the very end of the ramp stretch and the “static response” response measured at the end of the hold phase after the stretch. Several stretches were imposed during one contraction, generating a force record for each stretch. For each record, the dynamic and static force points were plotted against each other, and the force responses of a reinnervated muscle were compared with its untreated equivalent in the contralateral limb. During a crossed reflex action, the force in the activated muscles was the contribution from autogenic reflexes. The difference between the untreated muscle forces and the reinnervated muscle forces was the contribution from autogenic reflexes. The data were not pooled across animals. All of the off-line analysis was done with custom software written in Matlab from Mathworks.

RESULTS

The majority of measurements were made on the intact gastrocnemius muscle (G, 5 of 7 preparations). Some data were obtained for the surgically separated LG (2 of 7 preparations) and MG (1 of 7 preparations). However, because results obtained for LG and MG were similar to those of G, they were not distinguished for the final analysis. Compared with their untreated counterparts at matched initial forces, reinnervated muscles showed comparable initial stiffness because the initial force trajectories were the same in the two muscles. It is also apparent that the reinnervated muscles were associated with reduced $K_e$ and increased yielding (Fig. 2). In the untreated G muscle, the linearization by reflex action appeared to be more complete than in the untreated SOL muscle, and the force during the hold phase was more constant (compare dotted lines in Fig. 2, D and E, to that of A and B) over a range of initial forces. The force responses of G even more closely resembled the length input, thereby exhibiting the properties of a nearly ideal spring under these conditions. This pattern was observed in five out of six treated cats, with one preparation displaying a globally poor state of muscle activation and reflexes.

The force dependencies of the responses of injured and untreated muscles were quantified by plotting the responses at three time points against the corresponding initial forces (Fig. 3). The first point in time that was measured was the “prereflex” point (Fig. 3C). The prereflex measurement was collected 10 ms after the onset of the stretch as an assessment of the intrinsic properties of the muscles prior to any reflex action (Bonasera and Nichols 1994). The prereflex force response was not linearly correlated to initial force. In this example, the prereflex force response of the untreated muscle was higher (~2N) than that of the reinnervated muscle over the entire force range. Such small shifts were observed across animals but were not consistently skewed in a particular direction that

FIG. 2. Comparisons of reinnervated and untreated muscle responses to a constant velocity stretch (2 mm in 50 ms). Data shown are from 1 animal. • • • • untreated muscle responses with intact autogenic reflexes. — reinnervated muscle responses from treated limb. Records were superimposed at matched initial forces (number at left of traces). Left: gastrocnemius (G). Right: soleus (SOL). Top: comparison of autogenic and intrinsic muscle responses at high initial forces. Middle: similar comparison at low initial forces. Bottom: imposed length changes with time axis applicable to all traces above. Note the different calibration bars for the force traces of SOL and G. The G autogenic response was very linear and closely resembled the length input. The overall stiffness of both muscles was significantly increased in the presence of autogenic reflexes.
would suggest systematic differences in connective tissue between the reinnervated and untreated muscles.

The “dynamic” measurement was taken at the end of the ramp stretch, 50 ms after stretch onset (Fig. 3D). The increase in the dynamic response caused by autogenic reflexes was ~18.5 N in this example (average difference between dynamic response curves of reinnervated and untreated muscles in Fig. 3D). Across six different animals, the increase due to autogenic reflexes ranged from 6.50 to 19.04 N, for an average of 13.36 N. The third time point, labeled as “static” in Fig. 3, corresponded to the tonic stretch reflex (300 ms). The presence of autogenic reflexes increased the static response by an average of 15.3 N (average difference between dynamic response curves of reinnervated and untreated muscles in Fig. 3E) in this example (range from 8.98 to 24.07 N across all animals). The responses of untreated muscles (dynamic and static) showed little dependence on the initial force of the muscle, indicating that stiffness was regulated in those muscles. During the hold phase, the force responses of untreated G demonstrated relatively less decay than observed for untreated SOL responses. Due to this relative lack of adaptation, reflex action resulted in a substantial increase of muscular stiffness in G, even at higher forces.

The responses were also averaged over the time period from the onset of stretch to the end of the static phase 300 ms later. These averaged responses (Fig. 3F) further demonstrated stiffness regulation and substantial increases in $K_e$ by reflex action. Across all animals, autogenic reflexes added an average of 14.8 N-sec to the intrinsic static response (range from 8.87 to 22.73 N-sec).

The extent to which reflex action enhanced muscular stiffness varied considerably across preparations (Fig. 4). Relationships between dynamic response and force were pooled in one panel and static relationships in another panel. Polynomial curves were fitted to the data for the sole purpose of visually associating the data originating from each of the animals. The curves corresponding to reinnervated muscles ($E$ and $F$) were grouped at the bottom of each panel. As expected with the disruption of the autogenic reflexes, their values were lower than those of the untreated muscles ($D$ and $F$) within a given animal. The lines representing polynomial fits to the data from different reinnervated muscles were clustered together. Curves representing fits to data from untreated muscles were widely dispersed, suggesting large variations in reflex gain among preparations. In some cases, inputs from autogenic reflexes were very small. Note that the points corresponding to the dynamic responses of reflexive SOL (Fig. 4, top right) were less scattered than those of the static responses (Nichols 1974).
The reduced dispersion of responses from reinnervated muscles compared with untreated muscles led us to believe that the level of deafferentation was consistent throughout all animals (Fig. 4). Additionally, in the panels representing SOL, there was one animal that was acutely deafferented (△ and ▲). This complete deafferentation served as a control for the extent of reflex disruption of reinnervated SOL in the treated animals. The relatively tight clustering of data for reinnervated and deafferented SOL verifies the substantial loss of autogenic feedback in the reinnervated muscles.

We measured initial stiffness ($K_i$) and the extent of yielding ($K_e/K_i$) in the homogeneous SOL and heterogeneous G. $K_i$ of each muscle was evaluated as a function of the activation level. Measures of $K_i$, or those derived from $K_i$, were unaffected by autogenic reflexes as they occurred earlier in time. The initial stiffness ($K_i$) did not increase according to a straight-line relationship with the initial force ($F_i$) in either G or SOL, reinnervated or untreated. This was shown in Fig. 5 (A and B), top, for both reinnervated (left) and untreated (right) muscles in the same animal. Due to the longer fibers in SOL, we expected the $K_i$ of G to be almost twice that of SOL and the curves to progressively diverge. However, this expected difference was found only in some cases. For the untreated muscles shown in Fig. 5, the relationship of $K_i$ to $F_i$ was initially steeper for G in agreement with this prediction. However, as illustrated for the reinnervated muscles (Fig. 5, A and C), this difference was not always observed. The occurrence of differences in slope was not related to muscle treatment. As also illustrated in Fig. 5, the extents of curvature of the implied relationships varied considerably across muscles regardless of muscle species or treatment. The slopes of the $K_i$ versus $F_i$ relationships achieved plateaus at stiffness values roughly corresponding to the level of series compliance of the SOL tendon (Joyce et al. 1969).

As in homogeneous muscles, reflex compensation was significant in the untreated heterogeneous G, as indicated by increases in the ratio $K_e/K_i$ for both muscles (Fig. 6). In Fig. 6, top, data were obtained from reinnervated G and SOL. At low to moderate initial forces, $K_e/K_i$ was greater in G than in SOL. $K_e/K_i$ increased (indicating less yielding) with increased initial forces for both G and SOL and, interestingly, the differences between the values of G and SOL tended to diminish at higher force levels. In the bottom panel, we included results for the untreated muscles. For those muscles, values of $K_e/K_i$ exceeded 1 at low initial forces, indicating overcompensation by the autogenic stretch reflex. At higher $F_i$, the quantity $K_e/K_i$ of untreated muscles (● and ▽) tended to approach those of the reinnervated muscles (○ and ▼). For SOL, reflex action provided some compensation for the entire range of initial forces obtained in this experiment, whereas the responses of G be-
came dominated by intrinsic properties at relatively low levels of activation (Fig. 6B).

DISCUSSION

Three major findings resulted from this study. First, our results indicate that stiffness regulation is indeed a general function of proprioceptive feedback in both homogeneous and heterogeneous muscles. Stiffness regulation includes the compensation for yielding and a reduced dependence of overall muscular stiffness on initial force. Both components of stiffness regulation, which has previously only been shown in soleus muscle of the cat, were observed for the gastrocnemius muscle. Second, the relationship between initial stiffness and initial force saturates with increased levels of initial force for both muscles. Contrary to our predictions based on differences in motor-unit compositions of SOL and G, our prefl ex measure-ments of \( K_i \) in treated and untreated muscles do not reveal the expected significant differences in initial stiffness between these muscles except perhaps at very low forces. Architectural features and connective tissue are likely to be the most important determinants of this initial muscular response to stretch. Third, the extent of yielding, a feature that is strongly affected by the autogenic reflex and that therefore can only be investigated fully in the reinnervated preparation, is lower for G than for SOL as predicted according to the differences in fiber type composition. However, at higher forces, the yielding tendencies for both heterogeneous and homogeneous muscles approach similar values, with yielding tending to be less pronounced at higher activation levels. Additionally, this work extends the findings that the chronic reinnervation preparation is an effective means to eliminate autogenic reflexes for that muscle and that this reduction is independent of background force. This method, which allows the separation of intrinsic and reflex components of responses in homogeneous and heterogeneous muscles, facilitated the demonstration of the large range of reflex magnitudes that expressed across preparations.

Diminished reflex responses in reinnervated muscles

The autogenic afferent feedback to the motoneuron pool was dramatically diminished in the reinnervated muscles. Because some activity has been recorded from afferent fibers in a reinnervated muscle (Collins et al. 1986), the mechanism by which reflex activity was reduced in our preparations was not clear. Either the afferent fibers did not reinnervate their receptors (Dieler and Schröder 1990) or they were unable to form the appropriate synapses in the spinal cord to result in a reflex (Cope et al. 1994). The other possibility was that the afferents might re-innervate the receptors inappropriately such that Ib afferents that normally innervated Golgi tendon organs (GTO) reinnervated muscle spindles (Collins et al. 1986). However, force-dependent inhibition is also reduced in these preparations (Cope et al. 1994).

Intrinsic mechanical properties of reinnervated muscles: initial stiffness

Based on previous studies of single fibers and motor units (Malamud et al. 1996; Petit et al. 1990), we hypothesized that intrinsic properties of whole muscles could be strongly influenced by the type-specific properties of the active component motor units. Under this working hypothesis, we expected our measure of initial stiffness \( (K_i) \) to increase approximately linearly with contractile force for the homogeneous SOL. According to the hypothesis of Petit et al. (1990), we expected \( K_i \) to
increase according to a more convex relationship for G as more type II (and relatively more compliant) motor units were reinnervated. We were also interested in whether the intrinsic properties of reinnervated muscles might be altered by any changes in fiber type or motor-unit reorganization (Gillespie et al. 1987; Gordon and Stein 1982). However, the stiffness-force relationships for both muscles were all similarly convex and achieved plateau values in a similar range (10–15 N/mm). This finding suggests that fiber type is not the dominant factor in determining the force dependence of initial stiffness but is one of several factors that probably include architectural details and connective tissue elements. We will discuss each of these factors in turn.

In-parallel connective tissue most likely contributed to the vertical shifts of the stiffness-force relationships across muscles of a given species. While dissecting the muscles for terminal experiments, we noted adhesions in the operated limbs but carefully removed as many adhesions as possible along with in-parallel connective tissue. We attributed the vertical shifts to uncontrolled variations in the amounts of parallel connective tissue on the two sides. The upward shift of the stiffness-force relationships of G compared with those of SOL was likely due largely to greater in-parallel connective tissue in G, as indicated by the higher passive stiffness of this muscle (Walmsley and Proske 1981). In any case, connective tissue that was strictly in parallel with the muscle fibers was unlikely to affect the shape of the stiffness-force relationship. In contrast, in-series connective tissue would affect the shape of the stiffness-force relationship. At low forces, the stiffness of contracting muscle fibers and the tendon increase with force (Joyce et al. 1969; Rack and Westbury 1984). Ultimately, series compliance may limit muscular stiffness and therefore contribute to the decrease in slope of the stiffness-force relationship noted at higher activation levels. In addition, under a broad range of physiological conditions, series compliance, including tendon, would also allow some amount of internal shortening and reduction in stiffness (Houk et al. 1970; Sanderson 2000). One estimate of the relationship between series compliance and force for SOL (Joyce et al. 1969) indicated a maximum value, in units of stiffness, of ~14 N/mm. This value is close to the maximum value for the data reported here for both muscles. Previous evidence indicates that series compliance is similar for both muscles (Walmsley and Proske 1981). Finally, any connective tissue elements that would be slack in the passive muscle but involved by subsequent activation would influence the shape of the stiffness-force relationship. Of the known structures, series compliance appears to be the most likely connective tissue element responsible for the convexity of the stiffness-force relationship.

Pinnation angle and length of fibers are also architectural features that could influence the stiffness-force relationships. Changes in fiber orientation are relatively small in an activated muscle (Gans 1982; Sacks and Roy 1982), so pinnation angle is probably not a major factor. The length of the muscle fibers was likely to be a more important factor because the fibers of SOL are nearly twice the length of fibers in G (Sacks and Roy 1982). Indeed, the active stiffness of G is ~1.6 times that of SOL during tetanic contractions (Walmsley and Proske 1981). The shorter fiber lengths of G would be expected to result in a larger slope for the stiffness-force relationship of G, particularly at lower forces. Our failure to observe consistently larger slopes for G is unexplained. However, given the large variation in slopes of length-tension curves across animals (Nichols 1974), other factors could have obscured these differences.

There are also other factors that probably affect the shapes of the stiffness-force relationships of both muscles. First, it has been shown that the properties of type I motor units are not identical even in a homogeneous muscle like SOL (Cope et al. 1986). If the contractile kinetics of muscle fibers in larger type I motor units were faster than those of smaller type I motor units, then the initial stiffness of the muscle could obey a convex relationship to force (Huyghues-Despointes 1998) with respect to this factor as well. Second, the increases in firing rate that accompany recruitment (Cordo and Rymer 1982; Monster and Chan 1977) lead to larger increases in force than initial stiffness (Nichols 1974). This lack of proportionality would also have the effect of reducing the slope of the stiffness-force relationship at higher forces. Finally, some type II motor units are recruited at forces as low as 5–10 N in the medial gastrocnemius muscle (Tansey and Botterman 1996) so that the in-

**FIG. 6.** $K_i/K_F$ plotted as a function of $F_i$. Top: SOL values (○) were below G values (□) as expected because SOL tends to yield more than G. Both reinnervated G and SOL showed a modest increase in $K_i/K_F$ with increased $F_i$. Bottom: untreated muscle values (○ and □) were high as a result of stiffness regulation via autogenic reflexes. At increased $F_i$, untreated muscle $K_i/K_F$ values merged with the reinnervated (intrinsic) muscle values.
fluence of type II motor units are distributed more evenly than expected over the whole range of forces. This feature of recruitment would tend to diminish differences in the mechanical behavior of the two muscles as a function of recruitment level. In summary, the relationships between initial stiffness and force are most likely shaped by a complex interaction among contractile kinetics, series compliance, and fiber length. The similarity in the behavior of the initial mechanical response of the two muscles suggests that fiber type is not the dominant influence. The mechanical properties apparently result from a similar architectural feature such as series compliance or the cancellation of oppositely directed factors. Our results do not support the hypothesis that the different initial stiffness (prior to reflex effects) of type I and type II muscle fibers (Malamud et al. 1996) or motor units (Petit et al. 1990) strongly influence the mechanical properties of physiologically recruited muscles.

Intrinsic mechanical properties: tendency to yield

In contrast, the difference in tendency to yield between type I and type II motor units (Malamud et al. 1996; Stienen et al. 1992) did strongly influence the properties of physiologically recruited muscles, at least when comparing muscles of substantially different motor-unit composition. The tendency to yield was significantly greater in SOL than in G, indicating that this property was strongly dependent on fiber type. Surprisingly, however, the tendency to yield at higher activation levels declined in both muscles but most strongly in SOL. We expected our index \( K'_i/K_i \) to increase to a small extent in SOL due to increases in motor-unit firing rate (Joyce et al. 1969; Nichols 1974). We also expected the ratio \( K'_i/K_i \) to increase to a larger extent in G as more type II motor units were recruited and as firing rate increased. The more modest decrease in the tendency to yield in G indicated that factors other than fiber type were also important in determining the resultant mechanical properties.

The greater magnitudes of \( K'_i/K_i \) of G than SOL at lower forces are most likely explained by two factors. In-parallel connective tissue responds to stretch with a largely elastic response. This response adds to that of the whole muscle and therefore increases the magnitude of \( K'_i/K_i \). The greater in-parallel connective tissue of G compared with that of SOL would have therefore contributed to the observed difference. Additionally, the finding that some type II motor units are recruited at low forces in MG (Tansey and Botterman 1996) indicates that motor units with a small tendency to yield contribute over a wide range of forces. This factor could at least partially explain the greater magnitudes of \( K'_i/K_i \) values of G compared with SOL at low forces as well as the reduced slope of the relationship between \( K'_i/K_i \) and force.

The shorter fiber length in G indicates that, for similar stretch velocities for the two muscles, the fibers of G lengthen at higher rates. Because \( K'_i/K_i \) increases at higher velocities (Nichols 1974), fiber length is likely to have contributed to the observed differences in the tendency to yield between the two muscles. In summary, the greater magnitudes of \( K'_i/K_i \) for G are potentially explained by differences in fiber type, in-parallel connective tissue and fiber length. During walking and trotting, G undergoes active lengthening that is only \( \sim 25\% \) less than that for SOL due to movements at the knee (Goslow et al. 1973), so the effect of fiber length is compensated only partially during natural movements.

Reflex action

The stretch reflex modified the mechanical properties of both the soleus and gastrocnemius muscles to a major extent for a large range of initial forces. The extent of yielding was reduced according to our index, \( K'_i/K_i \). Although the magnitudes of this index were different for the intrinsic properties of the two muscles, these magnitudes were in the range 0.25–0.6 over the full range of forces. These values indicate that both muscles exhibited nonlinear behavior, at least for the experiments reported here in which stretches were delivered after a period of isometric contraction (see Huyghues-Despointes 1998; Nichols et al. 1999b). The magnitudes of this index in the presence of the stretch reflex were similar for both muscles and exceeded 1.0 at forces below \( \sim 10 \) N (Fig. 6B). The presence of the stretch reflex increased the magnitude of \( K'_i/K_i \) substantially up to the maximum force exerted by SOL \((\sim 30 \) N) and up to one half the maximum force exerted by G \((\sim 60 \) N). At higher forces in G, the magnitude of the \( K'_i/K_i \) index was in the range of 0.5–0.75 and no different from the tendency to yield of the reinnervated muscles. However, the reflex increased \( K'_i \) substantially at higher forces. To place these findings in functional perspective, we note that forces in SOL and the medial gastrocnemius muscle can each achieve 20 N during walking and trotting and during jumping forces in medial gastrocnemius can achieve \( \sim 80 \) N (Walmsley et al. 1978). Therefore between a reduction in yielding and enhancement of \( K'_i \), reflex action can potentially regulate the mechanical properties of both muscles for the full range of forces exerted during locomotion.

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