Synergistic and antagonistic interactions in the rat forelimb: acute effects of coactivation

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Maas H, Huijing PA. Synergistic and antagonistic interactions in the rat forelimb: acute effects of coactivation. J Appl Physiol 107: 1453–1462, 2009. First published September 10, 2009; doi:10.1152/japplphysiol.00328.2009.—The goals of the present study were 1) to assess effects of antagonist coactivation on mechanical interactions between synergistic muscles, and 2) to quantify the extent of epimuscular myofascial force transmission between synergistic and antagonistic muscles in the rat forelimb. Connective tissues enveloping the muscle bellies in the antebrachium were left intact. Forces exerted at the distal tendons of flexor carpi ulnaris (FCU), palmaris longus (PL), and extensor carpi ulnaris (ECU) muscles were measured at various FCU lengths for two different stimulation protocols: 1) simultaneous stimulation of ulnar/median nerve complex (exciting all wrist flexors, including synergistic FCU and PL) and radial nerve (exciting all wrist extensors, including antagonist ECU); and 2) stimulation of the ulnar/median nerve exclusively. PL and ECU were kept at a constant length. In addition, muscle forces were measured during stimulation of one of the indicated nerves, with later addition of stimulation of the second nerve during the maintained tetanic contraction. Coactivation of antagonistic muscles increased FCU isometric forces (on average, by 10% of optimal force) and PL forces (on average, by 13% of maximal force), but mechanical interaction between FCU and PL was unchanged. Changing the length and relative position of FCU significantly affected PL (by 13%) as well as ECU forces (by 8%). In addition, distal tetanic force of FCU kept at a constant high length was determined by the order of nerve stimulation onset. These results indicate effects of myofascial pathways between synergistic and antagonistic muscles in the rat forelimb. Coactivation may enhance the stiffness of connective tissues between muscles, but the present data suggest that activation of all wrist flexors already preloaded the myofascial pathways to the greatest extent. The stimulation order effects were explained by dynamic features of muscle and connective tissues (i.e., length-history dependence and viscoelasticity).

IN THE LAST DECADE, WE HAVE demonstrated the potential of force transmission between skeletal muscle and the surrounding connective tissues (for reviews, see Refs. 8–10, 17, 20). Such epimuscular myofascial pathways are responsible for mechanical interactions between synergistic muscles and, as shown more recently, also between antagonistic muscles (12). The extent of intermuscular interactions is dependent on the relative position of muscle bellies. During normal movements of the intact musculoskeletal system, synergistic muscles generally change length simultaneously. Changes in muscle relative position between synergistic muscles do occur, however, as a result of differences in moment arm (e.g., 14) and because some muscles span only one joint and other muscles span more than one joint. Note that any joint movement involves substantial changes in relative position between antagonistic muscles.

In contrast to our previous results (see above-cited review articles), Maas and Sandercock (19) recently found no mechanical interaction between the active one-joint soleus and passive two-joint ankle plantar flexors in the cat. In addition, others have reported that the whole fascicle length of soleus was not affected by changes in knee angle, as measured with ultrasound in both passive and maximally active conditions of the ankle plantar flexors (15). On the other hand, recent imaging studies in humans suggest that mechanical connections between gastrocnemius and soleus muscles are effective also within the in vivo length range. A twitch contraction of medial gastrocnemius muscle at a fixed angle of the ankle and knee joints elicited a decrease of fascicle length, not only in the excited muscle, but also in soleus muscle (24). Length changes within passive soleus muscle and within other muscles that do not cross the knee joint were also found with changes of knee angle, as assessed with MRI (32).

Up to this point, the effects of epimuscular pathways on muscular force transmission were tested predominantly during simultaneous maximal (e.g., Ref. 12) or submaximal (21) activation of both synergistic and antagonistic muscles. It is conceivable that effects of epimuscular myofascial force transmission may depend on how many muscles are active simultaneously. Coactivation may enhance the stiffness of the connective tissues and, thereby, facilitate force transmission between muscles. The lack of intermuscular interactions for knee joint movement in the cat found by Maas and Sandercock (19) may be explained by the absence of coactivation. Therefore, our first goal was to assess effects of antagonist coactivation on the mechanical interaction between synergists. The experimental procedures of Maas and Sandercock do not allow for testing the effects of coactivation, because nerve branches innervating synergistic muscles could not be kept intact. These experiments are a first step toward a better understanding of the effects of coactivation, which augments the existing data on epimuscular myofascial force transmission.

In most of our laboratory’s previous experiments in rat, muscles of the lower hindlimb were studied (e.g., Ref. 12). Hindlimb muscles in the rat (a quadruped) are involved predominantly in locomotion and in maintaining posture, while the forelimb muscles are also involved in fine motor tasks, such as prehension (30). As the architectural features of muscle (e.g., angle of pennation, fiber length) are known to be related to its function (26, 31), differences in connective tissue structure between muscle groups in the hind- and forelimb may also be expected. Therefore, the second goal of this study was to quantify the extent of epimuscular myofascial force transmis-
sion between synergistic and antagonistic muscles in the rat forelimb. The effects of surgical interventions (i.e., compartmental fasciotomy and partial muscle dissection) on the flexor carpi ulnaris muscle (FCU) have been studied previously in the rat (27). However, the effects on force transmission between FCU and adjacent muscles were not measured in that study.

Specifically, we tested the hypotheses 1) that the mechanical interaction between FCU and palmaris longus (PL; a synergist of FCU) is altered by coactivation of antagonistic muscles and 2) that isometric forces are transmitted via epimuscular myofascial pathways between FCU and PL, as well as between FCU and extensor carpi ulnaris (ECU; an antagonist of FCU). For that purpose, FCU, PL, and ECU isometric forces were measured at various muscle-tendon complex (MTC) lengths of FCU.

 METHODS

Animals. The data were obtained from five male Wistar rats (body mass 302 ± 16 g). Surgical and experimental procedures were in strict agreement with the guidelines and regulations concerning animal welfare and experimentation set forth by Dutch law, and approved by the Committee on Ethics of Animal Experimentation at the VU University Amsterdam.

Before the surgical preparations and data collection, the rats were deeply anesthetized using intraperitoneally injected urethane (initial dose 1.2 ml/100 g body mass, 12.5% urethane solution), as judged by complete absence of withdrawal reflexes. If withdrawal reflexes could be elicited, supplemental doses (0.3–0.5 ml/time) were administered. To prevent hypothermia, the animals were placed on a heated water pad at ~37°C. At the end of the experiments, the rats were euthanized without regaining consciousness with a lethal dose of pentobarbital sodium (200 mg ip) and double-sided pneumothorax.

Surgical procedures. The right forelimb was shaved, and the skin was resected from the shoulder to the wrist. The distal tendons of FCU, PL, and ECU were identified and dissected free from surrounding tissues in the most distal part of the antebrachial compartment. The more proximal connective tissues enveloping the muscle bellies were left intact.

With the wrist in a neutral position (i.e., 180° flexion, 0° ulnar deviation, 0° pronation) and the elbow joint at ~90°, markers were placed on the distal tendons of PL, ECU, and FCU muscles using 7–0 suture (Prolene, Ethicon). Subsequently, the PL and ECU tendons were cut from their insertion sites, and Kevlar threads (Goodfellow, Cambridge, UK) were tied to them for later attachment to the force transducer in the experimental setup. The FCU insertion was released by excising the pisiform bone from the carpus, leaving the tendon-bone connection intact, which was attached to Kevlar thread. It should be noted that, once a muscle is detached from its insertion, there is only a limited length range in which the muscle is at a physiological position relative to its surrounding muscles, if they are not changed in length. Further dissection was performed in the brachial compartment to secure a metal clamp to the humerus and in the antebrachial compartment to secure a metal clamp to the radius for later fixation in the experimental setup.

Within the brachial compartment, the ulnar, median, and radial nerves run parallel to the humerus on the medial side (for details, see Ref. 4). These nerves were identified and cut from the brachial plexus in the axilla, eliminating effects of spinal reflexes. Up to the point of entering the antebrachium, the ulnar and median nerves (U/M) are enclosed together in a sheath of epineurium. Therefore, they were dissected jointly. The radial nerve (R) was dissected up to the location of passing between the m. latissimus dorsi and m. cutaneus maximus to the brachium, approximately halfway down the humerus. The U/M innervate all palmar muscles of the antebrachium (“wrist and digit flexors”). Specifically, the U/M innervate FCU and PL muscles as well as m. flexor digitorum sublimis, m. flexor digitorum profundus, m. pronator quadratus, m. flexor pollicis, m. adductor pollicis, and m. epitrochleoanconeus (for anatomical description of these muscles, see Ref. 4). The R innervates all dorsal muscles of the antebrachium (“wrist and digit extensors”), as well as some brachial muscles. Specifically, the R innervates ECU muscle, as well as m. extensor carpi radialis, m. extensor digitorum communis, m. extensor digitii quarti, m. extensor digitii quinti proprius, m. supinator, m. abductor pollicis, m. extensor pollicis, m. extensor indicis proprius, m. triceps brachii, and m. anconeus (for anatomical description of these muscles, see Ref. 4). The musculocutaneous nerve was cut, denervating the biceps brachii and brachialis muscles.

Experimental conditions. The experimental setup (Fig. 1) was similar to the one used in a previous study performed in our laboratory (27). The rat was placed on a heated platform. The right forelimb was secured rigidly by clamping the humerus and radius, as well as by firmly tying the dorsal side of manus to an aluminum plate with 1–0 silk suture. The lower arm was secured in the horizontal position with the palmar side of the manus facing upwards, the wrist in neutral position, and the elbow joint at ~90°. With the use of the Kevlar threads in series with steel rods, the distal tendons were connected to three separate force transducers (BLH Electronica, Canton, MA; maximal output error <0.1%, compliance of 0.0162 mm/N), which were mounted on single-axis micropositioners. The space previously occupied by the pisiform bone was used to indicate the reference length of FCU. The marker on the FCU tendon was then aligned with the markers on the distal tendons of PL and ECU to indicate the

Fig. 1. Schematic view of experimental setup. The distal tendons of m. flexor carpi ulnaris (FCU), m. palmaris longus (PL), and m. extensor carpi ulnaris (ECU) were connected to three separate force transducers (F-FCU, F-PL, F-ECU). ECU was connected via a tube in between the plate and the dorsal side of the manus. The connective tissues enveloping the muscle bellies were left intact. The ulnar/median (U/M) and radial nerves (R) were identified and cut from the brachial plexus in the axilla, and each was placed on a pair of silver electrodes. The right forelimb was secured rigidly by clamping the humerus and radius, as well as by firmly tying the dorsal side of manus to a plate. The lower arm was secured in the horizontal position, with the palmar side of the manus facing upwards, the wrist in neutral position, and the elbow joint at ~90°. In the experiment, the length of FCU exclusively was varied [ΔL_{FCU}, FCU; where ΔL_{FCU} is muscle-tendon complex (MTC) length expressed as the deviation from optimum length (L_o)]. Drawing was made in Anim8or version 0.95 (www.anim8or.com).
reference length of these muscles. Note that this length corresponds to the relative position of the muscles during testing that resembles the relative position that would occur in vivo.

The force transducers were positioned in the line of pull for each muscle. The line of pull for ECU runs as close as possible to the dorsal side of the manus, which was obstructed by the forepaw plate. Therefore, we passed the Kevlar thread attached to the ECU tendon through a Teflon tube (inner diameter 0.8 mm) in between the plate and the dorsal side of the manus (Fig. 1). To assess the force loss in the tube due to friction, Kevlar thread was attached to a spring, passed through the tube, and connected to a force transducer at both ends (see also Ref. 18). The tube was fixed by a clamp. Various force levels were obtained by changing the distance between the force transducers. The tube introduced a small force leak (i.e., the difference in force between the two force transducers < 0.6% within the ECU force range measured).

The U/M nerve, as well as the R nerve were each placed on a pair of silver electrodes. The nerves were prevented from dehydrating by covering them with a thin piece of latex. In all experimental conditions, the nerves were stimulated supramaximally with the electrodes connected to a constant-current source (3 mA, pulse width 100 μs). Muscle temperature was controlled by airflow (Holland Heating) around the muscle of 22 ± 0.5°C and 80 ± 2% humidity. Dehydration of muscle and tendon was prevented by regularly irrigating these tissues with isotonic saline.

Experimental protocols. For all experimental conditions, the PL and ECU muscles were kept at the reference length. Before acquiring data, FCU was preconditioned by isometric contractions alternately at high and low lengths until forces at low length were reproducible to remove effects of previous activity at high length (11).

PL, ECU, and FCU isometric forces were measured at various FCU lengths. This MTC was lengthened distally with 0.5-mm increments, starting at active slack length to ~1 mm over optimum length (L_o). Before excitation, FCU was brought to the target length. Two twitches were evoked, followed by a tetanic contraction of the muscles (stimulation frequency, 100 Hz). Passive force was assessed by calculating the mean for the 100-ms time window just before the tetanic contraction. Total force was assessed by calculating the mean for the last 100 ms of the tetanic contraction (see Fig. 6), a time window was used to assess force, as opposed to a single time point. Force signals were acquired at 1 kHz and stored on a personal computer using a data-acquisition board (PCI-6221, National Instruments, Austin, TX). After each contraction, FCU was allowed to recover at low length for 2 min. Length-force data were collected for two different stimulation protocols: 1) simultaneous stimulation (pulse train 500 ms) of U/M and R nerves (Fig. 2A), and 2) stimulation of the U/M nerve exclusively (Fig. 2B). Control measurements were performed following the first and second set of length-force measurements to monitor any change in distally exerted muscle forces, but only minimal changes were found (<3% for FCU, <1% for PL, and <3% for ECU).

For selected FCU lengths (approximately L_o + 0.5 mm, L_o − 1.5 mm, and L_o − 3.5 mm), effects of two additional stimulation protocols were tested after the above-described experimental protocols. These protocols involved stimulation of the U/M and R nerves with pulse trains of unequal lengths (500 and 1,000 ms), adding stimulation of the second nerve while the first one remained stimulated (Fig. 2, C and D).

Treatment of force data and statistics. The individual MTC length-passive muscle force data were least squares fitted with an exponential curve (Eq. 1).

\[ y = e^{a_0t^a_1} \]  \hspace{1cm} (1)

where \( y \) represents passive muscle force, \( x \) represents MTC length, and \( a_0 \) and \( a_1 \) are coefficients determined in the fitting process. Active muscle force was assessed by subtracting passive force from total muscle force at equal MTC length. Length-active force data were fitted by a polynomial (Eq. 2).

\[ y = b_0 + b_1x + b_2x^2 + b_3x^3 + b_4x^4 + \ldots + b_nx^n \]  \hspace{1cm} (2)

where \( y \) and \( x \) represent active force and MTC length, respectively, and \( b_0 \) through \( b_n \) are coefficients determined in the fitting process. The order of the polynomial most adequately describing the data was selected, using a stepwise polynomial regression procedure. Fitted curves were used to calculate mean data and SDs, as well as to determine optimal force and \( L_o \). MTC length was expressed as the deviation from \( L_o \) (\( \Delta L_{o+} \)). Passive force exerted at the distal tendons of PL and ECU was negligible (< 0.01 N) in all experimental conditions, and, therefore, only the total forces are shown.

To test for effects of FCU length on PL, FCU, and ECU forces, one-way ANOVAs for repeated measures were performed. Effects of FCU length on PL and ECU forces at reference length were tested by one-way ANOVAs, including only the nearby data points (i.e., from \( \Delta L_{o+} = -3 \mathrm{~mm} \) to \( \Delta L_{o+} = -1 \mathrm{~mm} \). Two-way ANOVAs for repeated measures were used to analyze effects of R stimulation on FCU passive and active forces, as well as on PL force. If significant interaction effects were found, Bonferroni post hoc tests were performed to locate significant differences at specific MTC lengths of

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**Fig. 2.** Schematic representation of the different nerve stimulation protocols used. A: simultaneous tetanic stimulation of U/M and R nerves (results in Fig. 3). B: stimulation of U/M nerve exclusively (results in Figs. 4 and 5). C: stimulation of the U/M nerve, adding R nerve stimulation after 500 ms, while U/M nerve stimulation is continued (results in Fig. 6A). D: the same as in C, but starting with stimulation of the R nerve (results in Fig. 6B). The U/M nerve innervates all palmar muscles of the antebrachium (wrist extensors). The R nerve innervates all dorsal muscles of the antebrachium (wrist extensors). Vertical lines indicate twitches, and rectangles indicate the pulse train at 100 Hz. See METHODS for details.
FCU. A one-way ANOVA for repeated measures with a Bonferroni post hoc test was also applied to test for effects of nerve stimulation protocol (Fig. 2 A, C, and D) on FCU, PL, and ECU tetanic forces. Differences between ECU steady-state force during stimulation of the R nerve exclusively and ECU steady-state force during stimulation of R and U/M nerves simultaneously were tested by using paired t-tests. P values < 0.05 were considered significant.

RESULTS

Effects of changing length of FCU distally. For simultaneous stimulation of the U/M and R nerves, length-force characteristics of FCU, as well as results of simultaneously measured forces exerted at distal tendons of PL and ECU muscles, are shown in Fig. 3. Optimal active force of FCU was 3.60 ± 0.27 N, and L₀ was 32.9 ± 0.7 mm. FCU passive force at L₀ was 0.05 ± 0.02 N. Note the exertion of notable passive FCU forces at low lengths (ΔLₘₐₓ > -4 mm). FCU reference length is located 1.9 ± 0.7 mm below L₀. At this length, active force was 2.89 ± 0.22 N, and passive FCU force was 0.01 ± 0.004 N.

Lengthening of FCU distally yielded significant (ANOVA) force decreases at the distal tendon of PL (Fig. 3B). Note that PL was kept at a constant length (i.e., reference length). PL force decreased from its initial value of 0.98 ± 0.18 N at low-FCU length (ΔLₘₐₓ = -5 mm) to 0.78 ± 0.10 N at high-FCU length (ΔLₘₐₓ = 0.5 mm), a decrease of 20%. Effects of FCU length on PL force were significant also if only the data points near reference length (from ΔLₘₐₓ = -3 mm to ΔLₘₐₓ = -1 mm) were included in the statistical analysis. The major changes in PL force, however, occurred at low-FCU lengths (0.17 N between ΔLₘₐₓ = -5 and reference length).

Total force exerted at the distal tendon of antagonistic ECU, kept at a constant MTC length, was also affected significantly (ANOVA) by MTC length changes of FCU (Fig. 3C). Lengthening FCU over the full range resulted in a 8% decrease in ECU force, i.e., from 1.96 ± 0.34 to 1.81 ± 0.40 N. Changes in ECU force were more equally distributed over the range of FCU lengths tested (0.09 N between −5 and −2.5 mm, 0.05 N between −2.5 and 0 mm), but similar to PL significant near reference length (from ΔLₘₐₓ = -3 mm to ΔLₘₐₓ = -1 mm).

These results indicate that changing MTC length and relative position of FCU affects its distal tendon force and force transmission of synergistic PL, as well as of antagonistic ECU.

Effects of static coactivation of the dorsal muscles of the antebrachium. A similar experiment as described above testing effects of FCU length changes was performed, but with one major difference: only the U/M nerve was stimulated (U/M-only protocol, Fig. 2B). Thus the dorsal muscles of the antebrachium, i.e., the wrist extensors, including the ECU, remained passive.

ANOVA indicates significant main effects on FCU active force for FCU length and for stimulation protocol (U/M and R vs. U/M-only stimulation), as well as interaction between these factors. Thus excluding stimulation of the R nerve significantly altered FCU length-active force characteristics (Fig. 4A). Post hoc analysis showed that FCU active forces were significantly lower between ΔLₘₐₓ = -3.5 mm and ΔLₘₐₓ = -1.0 mm, which includes reference length. Optimal active force of FCU was 3.41 ± 0.26 N, and L₀ shifted to a higher length by 0.5 mm. Active force at reference length (ΔLₘₐₓ = -1.9 mm) decreased by 20%. The FCU length-passive force characteristics were similar to the first set of measurements (Fig. 4B).

ANOVA indicates significant main effects of FCU length and stimulation conditions on PL distal forces (Fig. 5). If only
Effects of dynamic coactivation of the dorsal muscles of the antebrachium. Figure 6 shows superimposed typical waveforms of FCU force for different stimulation protocols of the U/M and R nerves (shown schematically in Fig. 2). These protocols were applied for a constant high-FCU length, which, for this example, corresponds to 0.9 mm over \( L_0 \). If the R nerve was stimulated (exciting the wrist extensors) after the onset of U/M nerve stimulation (exciting the wrist flexors, protocol shown in Fig. 2C), an initial steep increase in FCU force was observed, followed by an exponential decay (Fig. 6A). This particular shape of the force waveform of FCU muscle tested at high length was observed in all animals.

Surprisingly, the mean FCU force at the end of nerve stimulation was substantially higher (3.60 N) than the mean FCU force (3.14 N) following synchronized onset of the U/M and R stimulation (protocol shown in Fig. 2A). This was also true if stimulation was continued for an additional 500 ms (\( n = 2 \), data not shown). Note that, in agreement with the results shown above (Fig. 4A), FCU force during stimulation of U/M nerve exclusively was lower (2.95 N) than if the R nerve was stimulated simultaneously. This is also true for PL force (\( n = 5 \), data not shown).

If the order of nerve stimulation onset was reversed (first R nerve then U/M nerve stimulation protocol shown in Fig. 2D), a small (0.11 N), but exponentially decaying force was found at the distal tendon of nonactive FCU during stimulation of the R nerve exclusively (Fig. 6B). Note that, in this condition, FCU force as well as its synergistic muscles are not excited. Therefore, this force originated most likely from antagonistic muscles and was transmitted via myofascial pathways to FCU. After the onset of U/M nerve stimulation, FCU total force increased and attained a steady-state level. In contrast to the other stimulation protocol, FCU force was lower (3.01 N) than following simultaneous stimulation of U/M and R (3.14 N).

These typical results suggest that, at high length, the force exerted at the distal tendon of FCU is dependent on the activity of antagonistic muscles, as well as on the order of nerve stimulation.

Mean tetanic FCU forces at the end of nerve stimulation for the different protocols are shown in Fig. 7. The results for high

![Graph](image-url)
as well as low and moderate lengths are presented. We found a significant main effect of stimulation protocol at high-MTC length (ANOVA), statistically confirming for all animals (n = 5) the typical example described above. Particularly, it should be noted that active FCU force found for U/M nerve stimulation followed by R nerve stimulation (total force = 3.94 ± 0.34 N, see high-FCU length in Fig. 7; passive force = 0.05 ± 0.01 N) was higher than optimal active force found for the static U/M and R simultaneous stimulation condition (3.60 ± 0.27 N, see Fig. 3A). For the other two lengths tested, no significant stimulation history effects on FCU force were found (Fig. 7). Also for ECU and PL kept at reference length, the various stimulation protocols at the selected FCU lengths did not yield significant steady-state force differences (n = 5, data not shown).

The stimulation protocol that started with R nerve stimulation (exciting the wrist extensors, protocol shown in Fig. 2D) also yielded data on acute effects of coactivating the palmar muscles in the antebrachium (by U/M nerve stimulation) on ECU force (Fig. 8A). For two of the three FCU lengths tested, ECU steady-state force was significantly higher during stimulation of the R nerve exclusively than during stimulation of R and U/M nerves simultaneously (Fig. 8B). This suggests that, for these FCU lengths, part of force exerted at the distal tendons of FCU and PL during simultaneous stimulation of U/M and R nerves (protocol shown in Fig. 2A) originated from ECU muscle fibers.

We conclude that distally exerted force of FCU kept at a constant high length is codetermined by the order of nerve stimulation. We also showed that coactivation of the dorsal muscles (by R nerve stimulation) can acutely change FCU force, and that coactivation of the palmar muscles (by U/M nerve stimulation) affects ECU force. These results suggest force transmission from active sarcomeres to the tendons of active and passive antagonistic muscles.

DISCUSSION

This is the first study that investigated whether mechanical interactions between synergistic muscles (specifically FCU and PL) are affected by coactivation of antagonistic muscles. In contrast to our expectations, no significant effects were observed. However, interesting new effects of the timing of antagonist coactivation on FCU force were found. Our second aim was to quantify the extent of mechanical interaction due to epiuscular myofascial force transmission between synergistic and antagonist muscles in the rat forelimb. In agreement with previous data for hindlimb muscles (for an overview, see Ref. 3).
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8), we found that synergistic PL as well as antagonistic ECU forces were affected by changing the length and relative position of FCU.

There are several connective tissue structures within the rat forelimb that could have provided the mechanical linkage between synergistic and antagonistic muscles: 1) connective tissue at the muscle belly interface providing direct intramuscular connections between FCU and PL; 2) connective tissue that reinforces the neurovascular tract near the FCU, consisting of the dorsal and palmar branches of the ulnar nerve and the ulnar artery and vein (4); 3) the antebrachial compartmental fascia. Disrupting these connective tissues and partially separating FCU muscle from the neurovascular tract has been shown to affect force transmission from FCU (27). However, the effects on mechanical interactions between FCU and adjacent muscles were not measured in that study. In addition, the distal portion of the triceps brachii covers the origin of the superficial wrist extensors, such as ECU (29), which could have provided a mechanical connection with the also excited brachial muscles.

**Does muscle coactivation affect mechanical interactions between synergistic muscles?** Up to this point, two types of muscle excitation conditions have been used primarily to assess effects of epimysial pathways on muscular force transmission: 1) simultaneous activation of both synergistic and antagonistic muscles (e.g., Ref. 12); and 2) activation of a single muscle (19). These conditions appear to be extreme cases, but have been found in the awake, freely moving animal. Coactivation of synergic and antagonistic muscles has been reported to occur frequently during reaching movements in the cat (7, 28). Application of the first condition revealed clear myofascial effects, while this was not observed in the latter one. In the present study, we tested the hypothesis that the stiffness of the connective tissues (i.e., the myofascial pathways), and, thereby, the extent of intermuscular mechanical interactions, is modified by the number of muscles that are active simultaneously. The present results add another piece to this puzzle. Not activating the wrist extensors did not significantly alter the mechanical interaction between two wrist flexors (i.e., the slope of the PL force-FCU length curve was similar in both conditions, Fig. 5). This suggests that simultaneous activity of all palmar muscles in the antebrachium already preloads myofascial pathways maximally. Therefore, future experiments should further study effects of decreasing the number of muscles that are active simultaneously by also excluding activity of some synergic muscles.

**Force transmission between antagonistic muscles.** Force levels of FCU and PL at all FCU lengths tested were affected significantly by activity of the wrist extensors (Figs. 4 and 5). Note that this was also the case at reference length, suggesting effects at the in vivo length and relative position of all muscles. Isometric forces exerted at the distal tendons of FCU and PL were significantly higher when the wrist extensors were also activated by stimulation of the R nerve. This suggests that some force is transmitted from sarcomeres in the coactivated wrist extensors to FCU and PL. This is confirmed by our finding that ECU force decreased acutely, if U/M nerve stimulation was added during contraction due to R nerve stimulation (Fig. 8). Stimulation of the R nerve also excites some muscles in the brachium (see METHODS). Together with the observation that U/M nerve stimulation causes movement of superficial tissues in the brachium (e.g., distal aponeurosis of triceps brachii), this suggests that part of the additional force may originate from brachial muscles. As the ulna itself could not be fixed to the experimental setup (see Fig. 1), effects of coactivation may have been confounded by movements of this bone. We performed an additional experiment to assess ulnar translation and/or rotation by inserting a needle into the ulna. Coactivation of the wrist extensors by stimulation of the R did not change the position of the ulna in proximal or distal direction, but resulted in a small rotation (−5°) in a plane transverse to the ulna. However, relative movement (rotation) of ulna and radius during pronation and supination has been shown to have only minor effects on FCU force (16) and is thus
not likely responsible for the substantial increases in FCU force following antagonistic coactivation (i.e., up to 0.61 N, 35%; see Fig. 4).

**History effects of coactivation.** For the highest FCU length tested, we found that FCU force during simultaneous stimulation of both U/M and R nerves depended on the order of onset of nerve stimulation (Figs. 6 and 7). If the R nerve was stimulated after the onset of U/M nerve stimulation, FCU force was significantly higher (by 10%) than following synchronized stimulation of U/M and R nerves. In contrast, a lower FCU force (by 3.4%) was found, if the order of sequential nerve stimulation was reversed (first R nerve then U/M nerve stimulation). These results are remarkable because, at the instant FCU muscle force was determined in all three cases, stimulation conditions of U/M and R nerves are identical; only their acute previous stimulation history differed. Also, no changes in positions of forepaw, radius, and humerus could be detected between the three cases, and any differences in ulna rotation were minimal (<0.7°). It is concluded that skeletal movements due to ineffective fixation did not confound these comparisons. Therefore, the FCU force differences must be explained by mechanisms that are active during the dynamic phase of the coactivation, of which the effects persist during the final isometric phase.

Note that the results resemble those of isometric steady-state muscle forces found following either shortening or lengthening contractions (1, 5, 22, 23). In addition, the finding (for U/M + R stimulation) that FCU force attained values higher than its optimal force of the exclusively isometric condition is in agreement with the concept of lengthening-induced force enhancement (3, 25). Shortening-induced force depression and lengthening-induced force enhancement are reported at the muscle level, but also at the levels of muscle fiber and myofibril (6). It is important to point out here that our results were obtained during contractions at a constant MTC length. It is unlikely that relatively small length changes of the muscle belly, due to elastic length changes of the tendon, will cause effects of the magnitude observed, if it would be imposed equally along the length of the muscle fibers. However, a contribution of length-history effects is conceivable, if shortening or lengthening contractions are imposed locally on parts of the muscle fibers (i.e., imposed on a small fraction of sarcomeres arranged in series within a muscle fiber). Then relatively high strains will be found locally.

At the high FCU length for which these force differences were found, FCU muscle belly is positioned distally relative to its surrounding muscles. In such a relative position, the epimyscular connective tissues exert a proximally directed force on the FCU muscle belly. R nerve stimulation after the onset of U/M nerve stimulation will cause contraction of the wrist extensors. Such a contraction involves some shortening of the extensor muscle bellies (i.e., movement of its distal parts in proximal direction), causing further stretching of the connective tissue linkages with FCU and, consequently, enhancing the force exerted on the FCU muscle belly. This load on FCU will lengthen locally part of its already active muscle fibers, (i.e., sarcomeres within corresponding muscle fiber segments, causing force enhancement). Finite-element modeling studies indicate such local lengthening due to external (myofascial) pulling forces exerted on a muscle belly (e.g., Ref. 33). Note also that the peak in the force signal immediately after the onset of R nerve stimulation ($t = 1,600$ ms in Fig. 6A) is compatible with force signals found following lengthening contractions (2).

In addition to the mechanical interactions between FCU and the wrist extensors, part of the effects may also be the result of mechanical interactions between FCU and the muscles in the brachium that are also activated by stimulation of the R nerve (see METHODS). In a similar fashion, these muscles may also have loaded the muscle belly of FCU, actively lengthening its muscle fibers. As the vast majority of muscles activated by the R nerve are located in the antebrachium (see METHODS), it is expected that these muscle have contributed most to our results.

Similar phenomena but with opposite effects may be active if the R nerve is stimulated first. Again, initially the epimyscular connective tissues exert a proximally directed force on the FCU muscle belly, but in this case the wrist extensors are already active. Added U/M nerve stimulation will result in a contraction of the wrist flexors, including FCU muscle. In contrast to the condition described above, FCU muscle fibers shorten in the same direction as the force of the epimyscular linkages (i.e., proximal), enhancing the magnitude of local muscle fiber shortening (i.e., enhanced shortening of sarcomeres in series within muscle fiber segments, causing force depression).

![Fig. 9. Schematic of muscle fibers illustrating potential effects of myofascial loads on the difference between global and local length changes ($\Delta L$).](http://jap.physiology.org/)

A: Isolated fiber

- Proximal
- Distal

\[ \Delta L_{\text{global}} = \Delta L_{\text{local}} \]

\[ \pm 25\% \quad \pm 25\% \quad \pm 25\% \quad \pm 25\% \]

B: Fiber within connective tissue network

- Proximal
- Distal

\[ \Delta L_{\text{global}} \neq \Delta L_{\text{local}} \]

\[ 0\% \quad 0\% \quad +50\% \quad +50\% \]

Myofascial loads

C: Fiber within connective tissue network

- Proximal
- Distal

\[ \Delta L_{\text{global}} \neq \Delta L_{\text{local}} \]

\[ -8.3\% \quad -8.3\% \quad -8.3\% \quad \pm 25\% \]

Myofascial load

Fig. 9. Schematic of muscle fibers illustrating potential effects of myofascial loads on the difference between global and local length changes ($\Delta L$).

- A: if an isolated muscle fiber is stretched by 25%, this $\Delta L$ will be distributed equally among its elements in series. Thus each element is stretched also by 25%.
- B: global $\Delta L$ may be distributed unequally over its elements in series for a muscle fiber that is mechanically linked to its connective tissue stroma. This occurs if a myofascial load is not exerted (equally) on all elements in series. Thus a 25% stretch of the whole fiber could result in local $\Delta L$ that are higher than 25%.
- C: myofascial loads exerted on part of the muscle fiber may also result in a length increase of some fiber segments and a length decrease in other segments of the same fiber, while the global length remains unchanged. In the example, one segment increases length by 25%, while the other three segments in series decrease by 8.3% each (i.e., one-third of 25%).
Such dynamic effects of myofascial force transmission have not been described previously. Although the above explanation is a hypothesis, it suggests that the effects of stimulation history could be mediated by length-history effects. For a quantitative analysis, it is necessary to determine whether the changes in relative muscle position due to muscle contraction and, consequently, lengthening of the in-series elastic structures resulted in length changes of muscle fibers comparable to those imposed on maximally dissected muscles to indicate length-history effects (1, 5, 22). For comparison, shortening rat medial gastrocnemius muscle by 1 mm (its MTC length is comparable to the MTC length of FCU, ~30 mm) decreased isometric force at $L_o$ by ~5% (23). Lengthening the same muscle by 2 mm increased isometric force at $L_o$ by ~6% (22).

In an isolated muscle fiber, global length changes will be distributed equally among its elements in series (i.e., sarcomeres). In contrast, a muscle fiber that is mechanically linked to its connective tissue stroma may distribute global length changes unequally over the sarcomeres. In addition, myofascial loads exerted on part of the muscle fiber may result in a length increase in sarcomeres of some fiber segments and a length decrease in others of the same fiber, while the global length remains unchanged. Such effects of myofascial loads on the difference between global and local length changes are illustrated schematically in Fig. 9. Accordingly, length changes of sarcomeres locally within muscle fibers may be much higher than the global length changes imposed at the fiber level (e.g., Ref. 33). Consequently, length-history effects larger than expected from imposed global length changes may be present also in our study.

For the experimental procedures applied in the present work, it is currently not possible to assess the local length changes of FCU muscle fibers or sarcomeres within muscle fibers. Recent application of MRI image analysis technique to assess local in vivo deformation within human muscles indicates considerable serial strain distributions within muscle fibers and thus is promising in this regard (32).

In conclusion, forces in muscles with synergistic mechanical effects are affected by the timing of onset of activity of their antagonistic muscle(s) in a way that is compatible with local dynamic contractions of muscle fiber segments and their sarcomeres.

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