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Ciliary neurotrophic factor prevents unweighting-induced functional changes in rat soleus muscle

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Fraysse, B., C. Guillet, C. Huchet-Cadiou, D. Conte Camerino, H. Gascan, and C. Léoty. Ciliary neurotrophic factor prevents unweighting-induced functional changes in rat soleus muscle. *J Appl Physiol* 88: 1623–1630, 2000.—The purpose of the present work was to see whether changes in rat soleus characteristics due to 3 wk of hindlimb suspension could be modified by ciliary neurotrophic factor (CNTF) treatment. Throughout the tail suspension period, the cytokine was delivered by means of an osmotic pump (flow rate 16 $\mu g \cdot kg^{-1} \cdot h^{-1}$) implanted under the hindlimb skin. In contrast to extensor digitorum longus, CNTF treatment was able to reduce unweighting-induced atrophy in the soleus. Twitch and 146 mM potassium (K) tensions, measured in small bundles of unloaded soleus, decreased by 48 and 40%, respectively. Moreover, the time to peak tension and the time constant of relaxation of the twitch were 48 and 54% faster, respectively, in unloaded soleus than in normal muscle. On the contrary, twitch and 146 mM K contracture generated in CNTF-treated unloaded and normal soleus were not different. CNTF receptor-α mRNA expression increased in extensor digitorum longus and soleus unloaded nontreated muscles but was similar in CNTF-treated unloaded muscles. The present results demonstrate that exogenously provided CNTF could prevent functional changes occurring in soleus innervated muscle subject to unweighting.

ciliary neurotrophic factor receptor-α; mammalian skeletal muscle; hindlimb unweighting

HINDLIMB SUSPENSION HAS BEEN shown to produce similar biochemical and physiological changes in skeletal muscles occurring during spaceflights (27, 36). Muscle atrophy and functional alterations, which occurred in the tail-suspended rat model, are particularly evident in slow-twitch muscles such as the soleus (6). One of the most common observations made on suspended soleus is a shift in isometric twitch kinetic characteristics toward those of fast-twitch muscles such as extensor digitorum longus (EDL) (11, 18).

The functional characteristics of adult skeletal muscle depend on neuronal influences mediated by electrical activity and myotrophic substances (16). Neuronal electrical activity influences have been extensively studied (13, 21, 23, 29). However, until recent advances, it has been difficult to identify putative neurotrophic substances affecting contractile muscle properties. The ciliary neurotrophic factor (CNTF) was largely characterized by its ability to sustain the survival of motoneurons in vitro and in vivo (1, 28). This cytokine is abundantly synthesized by Schwann cells in adult peripheral nerves (12, 32) and uses a multimeric receptor (19). CNTF shares, with members of the cytokine interleukin-6 family, the transmembrane signal transducing proteins glycoprotein-130 and leukemia inhibitory factor receptor. The CNTF receptor also includes a specific binding subunit known as CNTF receptor-α (CNTFR-α) (5). Because CNTFR-α is required for signaling and defines potential CNTF targets, expression and tissue distribution of CNTFR-α largely govern the CNTF response (20). It has been demonstrated that CNTFR-α is expressed in skeletal muscle (5, 17, 24). Moreover, it has been shown that subcutaneous injections of CNTF reduce the denervation-induced atrophy and aging-induced strength decrease in skeletal muscles (14, 17).

We have previously shown that CNTFR-α expression increased in muscle after 3 wk of hindlimb suspension and returns to normal within 2 wk of recovery (15). This led us to the hypothesis that skeletal muscle after hindlimb unweighting represents a specific site for CNTF action. The aim of this study was to assess the ability of CNTF, exogenously provided, to prevent atrophy and functional changes induced by 3 wk of unweighting in rat skeletal muscles.

MATERIALS AND METHODS

General procedures. Animal care was consistent with the recommendations of the Helsinki Declaration, and the study was performed in accordance with regulations of the French Ministry of Agriculture. Before in vitro experiments, animals were heavily anesthetized by an ether vapor flow. After respiratory arrest, the EDL and soleus muscles were excised, weighted, and placed in oxygenated HEPES-buffered physiological saline at room temperature. The tendons of the muscle were then pinned in the dissecting dish. Each muscle was dissected into two parts lengthwise under a binocular microscope. One part was rapidly frozen in liquid nitrogen and stored at −80°C for subsequent molecular biology and

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biochemistry experiments. The other part was used for the contractile experiments.

Simulated microgravity and CNTF treatment. Adult male Wistar rats (3 mo old, weighing 305 ± 21 g) were used. A first group was subjected to 21 days of tail suspension by using a modified Morey's model (11, 25). The first group and a group of normal weight-bearing rats were treated by subcutaneous CNTF administration. The cytokine was slowly delivered by using an osmotic pump (model 2004, Alza, Palo Alto, CA). The pump was implanted under the left hindlimb skin of the animal, close to the muscles of interest. These animals were compared with control (Con) rats (without CNTF treatment, nonsuspended, age-matched, and housed in the same environment). Rat CNTF was produced as a glutathione *S*-transferase fusion protein by using the pGEX-4T2 gene fusion vector from Pharmacia (Uppsala, Sweden) and was further purified by means of a glutathione *S*-transferase purification module (Pharmacia). Bioactivity of CNTF was monitored in vitro by TF1 cell bioassay as described previously (3). Helgren and collaborators (17) tested the effect of daily injections of varying CNTF doses on denervation-induced skeletal muscle atrophy. According to their results, we used the smallest dose (0.3 mg/kg) for which a reduced denervation-induced atrophy was observed. Sterile filtered CNTF was diluted in PBS to fill up the pump with the appropriate volume and concentration to ensure a flow rate of 16 $\mu g \cdot kg^{-1} \cdot h^{-1}$ of the cytokine throughout the suspension period.

Tension measurements. Bundles 200 μm in diameter (3–5 fibers) were isolated from tendon to tendon, transferred on a coverslip in a drop of physiological solution, and mounted into an experimental chamber. Briefly, each end of the muscle was carefully snared by fine platinum wire loops: one fixed in the experimental dish and the other to the tip of a force transducer (displacement measuring system, Kaman KD 2300, Kaman, Colorado Springs, CO). The flow rate of solution in the experimental chamber was 20 ml/min. The preparation was stretched step by step to a length at which maximal developed twitch was obtained. Twitches were generated by pulses at twice the threshold amplitude delivered at a frequency of 0.1 Hz (platinum electrodes; ISOSTIM A320 stimulator, World Precision Instruments, New Haven, CT). In fast- and slow-twitch muscles, raising potassium (K) concentration in perfusing medium above a certain threshold leads to the development of a phasic contracture. K contractures obtained by the use of 146 mM K solution were reproducible over time. Therefore, to provide a basis for comparing data with our previous work and for preserving muscle preparations in experiments, 146 mM K contractures were used. After the first 146 mM K contracture (conditioning K contracture), the muscle is returned to the control solution; a second exposure to high-K solution then induces a reduced response (test K contracture). The repriming time course of the test K contracture amplitude can be determined by varying the time between the conditioning and test exposures with high-K medium. When this approach is used, the strength of the K contracture test showed a sigmoidal recovery with time. The repriming curve was fitted by means of a Boltzmann equation: $T = \{100/[1 + \exp(R - R_{50})/k]\} + 100$ (where T is the test K contracture-to-conditioning K contracture tension ratio, expressed as a percent; R corresponds to the time of recovery; R_{50} is the time required for 50% tension recovery; and k is a slope factor). At the end of each experiment, the diameter of the bundle was measured by using a binocular micrometer (×60 magnification). Before it was discarded, the muscle preparation was exposed to Triton X-100 diluted in a normal physiological solution. In these conditions, muscle fibers elicited an irreversible contracture (Triton contracture) after ~1 min of perfusion. Triton X-100 is known to make holes in all cellular membranes because of the myofibrillar machinery (37). As calcium is free to enter the cells and interact with contractile proteins, Triton contracture tension was defined as the maximal tension that the preparation could develop in our experimental chamber. Twitches and contractures were recorded on a chart recorder (SE 120, BBC Goertz Metrawatt, Nürberg, Germany) and stored in a computer (PC DX486) for further analysis by means of an acquisition card (Digidata Card 1200B, Axon Instruments, Foster City, CA). The constant of relaxation of twitches and K contractures was calculated by fitting the relaxing phase with a monoexponential function by means of a computer fitting software (Clampfit 6.0, Axon Instruments).

Solutions. The normal physiological solution contained (in mM) 140 NaCl, 6 KCl, 3 $CaCl_2$, 2 $MgC1_2$, 5 glucose, and 5 HEPES (all chemical compounds from Merck, Darmstadt, Germany, except HEPES from Sigma Chemical, St. Louis, MO). The pH was adjusted to 7.4 with Tris (Sigma Chemical). A depolarizing solution (146 mM K) was prepared by replacing a given amount of NaCl with KCl, and the $[K]_o \times [Cl]_o$ product (where brackets and subscript o denote extracellular concentration) was kept constant by replacing chloride with L-glutamate. Triton X-100 (Sigma Chemical) solution was prepared by diluting the detergent 100 times in the normal physiological solution. All experiments were performed at room temperature (19–21°C).

Total protein content determination. Pieces of frozen muscles were weighted and mechanically disrupted in 10 vol of PBS. The concentration of homogenates was determined with the Bio-Rad Protein Assay (Bio-Rad, Hercules, CA). Results are expressed as a ratio of milligrams of protein to 100 mg of muscle.

Northern blot analysis. Total RNA from soleus and EDL muscles was prepared with guanidium thiocyanate according to the procedure of Chomczynski and Sacchi (4). After dissolution in water, RNAs were denatured at 50°C during 1 h in glyoxal-DMSO buffer. Migration was performed in phosphate buffer, and the transfer was done by capillary on a nylon membrane. RNAs were then covalently linked to the membrane by ultraviolet fixation. The cDNA encoding CNTFR-α was kindly provided by G. D. Yancopoulos (Regeneron, Tarrytown, NY). Hybridization with CNTFR-α or glyceraldehyde-3-phosphate dehydrogenase ^{32}P-labeled cDNA probes was performed in Quikhyb buffer (Stratagene, La Jolla, CA) according to the manufacturer's instructions. The membranes were then exposed to autoradiographic films for 3 days before RNA levels were measured by densitometry. The CNTFR-α-to-glyceraldehyde-3-phosphate dehydrogenase signal ratio was computed.

Statistical analysis. All values are expressed as means ± SE. The number (n) of muscles or rats used in experiments is indicated in parentheses. For contractile property determination, at least two preparations by muscle were studied. Statistical analysis was carried out by using unpaired Student's t-test, and $P < 0.05$ was taken to reflect a significant difference between values measured in two populations of preparations.

RESULTS

Rat body weight during suspension and CNTF treatment. Before the 21-day period, all animals weighed 305 ± 9 g. After 21 days in normal weight-bearing conditions, body weights of CNTF-treated and Con animals did not differ ($P > 0.05$) and were 354 ± 7 g ($n = 5$) and 358 ± 9 g ($n = 8$), respectively. The body

weight of the CNTF-treated suspended group was unchanged after the suspension period (301 ± 7 g; $P > 0.05$; $n = 8$) but was ~15% lower than that in the age-matched normal loaded group ($P > 0.05$).

Suspension-induced atrophy in skeletal muscles. Hindlimb unweighting resulted in a progressive loss of muscle mass. This weight loss reaches a plateau after 3 wk of suspension and affects principally slow-twitch muscles such as soleus. This was expressed as a ratio of muscle weight (mg) to body weight (g). This ratio was reduced by ~50% in hindlimb unweighted (HU) soleus (muscle of unloaded treated rat excised in the right hindlimb where no pump was implanted) (Fig. 1*A*). On the other hand, the CNTF-HU soleus (muscle of unloaded treated rat excised in the left hindlimb where a CNTF delivering osmotic pump was implanted) muscle-to-body weight ratio was larger (22%) than that of the HU muscle (Fig. 1*A*). Conversely, the muscle-to-body weight ratio in CNTF-treated soleus of normal loaded rats (CNTF-Con) did not differ from values of Con muscles [0.55 ± 0.04 mg/g ($n = 5$) vs. 0.56 ± 0.03 mg/g ($n = 8$); $P < 0.05$]. Additionally, no significant change was observed in the ratio of muscle to body weight in HU, CNTF-HU, and Con EDL (Fig. 1*B*).

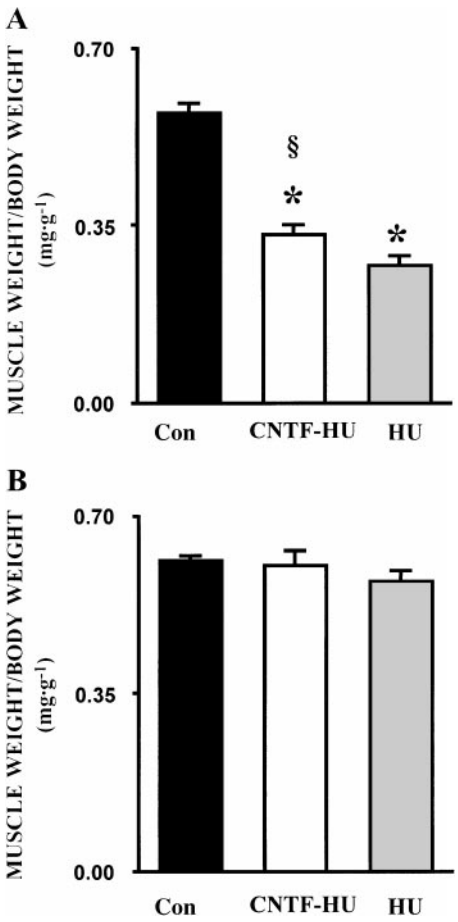

Fig. 1. Muscle weight-to-body weight ratio of soleus (*A*) and extensor digitorum longus (EDL; *B*) muscles. Con, control; CNTF, ciliary neurotrophic factor; HU, hindlimb unweighted. Con group, muscle of normal weight-bearing rats ($n = 8$, for EDL and soleus); CNTF-HU group, CNTF-treated unloaded muscle ($n = 8$, for EDL and soleus); HU group, contralateral unloaded muscle ($n = 8$, for EDL and soleus). Values are means ± SE. Significantly different from *Con soleus and §contralateral muscle, $P < 0.05$.

An additional measurement employed to monitor atrophy is the total muscle protein. The values for soleus were 6.8 ± 0.6 ($n = 5$) and 8.9 ± 0.7 mg protein/100 mg muscle ($n = 5$) for HU and CNTF-HU, respectively (significantly different $P < 0.05$), and 13.8 ± 1 mg protein/100 mg muscle ($n = 5$) for Con muscle (significantly different $P < 0.05$, HU vs. Con and CNTF-HU vs. Con). These results show that the total protein amount is significantly larger in the CNTF-HU soleus than in HU muscle. The total muscle protein content in HU EDL was slightly reduced compared with that in Con muscle. However, no significant difference was found in values of total muscle protein content of HU (11.5 ± 1 mg protein/100 mg muscle, $n = 5$), CNTF-HU (12 ± 2 mg protein/100 mg muscle, $n = 5$), and Con (16 ± 2 mg protein/100 mg muscle, $n = 5$) EDL ($P > 0.05$, HU vs. CNTF-HU, HU vs. Con, and CNTF-HU vs. Con).

Expression of CNTFR-α mRNAs. We have previously shown (15) that the expression of CNTFR-α mRNA in soleus muscle increased by ~2 after 3 wk of suspension. Therefore, a series of experiments was carried out to determine whether the administration of CNTF could restore normal CNTFR-α mRNA expression in suspended soleus. The mRNA expression of the specific receptor component for CNTF increased by 100% in HU soleus after 3 wk of suspension (Fig. 2*A*). Conversely, no significant difference was observed in the expression of CNTFR-α in CNTF-HU soleus compared with that in Con muscle. Approximately the same profile of mRNA expression was found in EDL muscle (Fig. 2*B*). However, in CNTF-treated unloaded EDL, the expression of CNTFR-α mRNA was not completely restored to its normal level.

Twitch characteristics. The isometric twitch tension, obtained from HU soleus, significantly decreased by 48% from Con values (Fig. 3, Table 1). Conversely, the twitch tension value of CNTF-HU unloaded soleus was significantly larger than that in HU muscle but did not differ from that in Con soleus (Fig. 3, Table 1). In CNTF-Con soleus, the isometric twitch tension was not different from the Con value (Table 1). The twitch tension was similar in HU, CNTF-HU, and Con EDL (Fig. 3, Table 1).

The twitch time to peak tension and the time constant of relaxation were 48 and 54% faster, respectively, in HU soleus than in Con muscle and were close to Con EDL twitch characteristics (Fig. 3, Table 1). By contrast, the time to peak and the time constant of relaxation of the CNTF-HU soleus were longer than those of HU muscle (Fig. 3, Table 1). Moreover, no difference was found between CNTF-HU and Con soleus (Fig. 3, Table 1). On the other hand, in CNTF-Con soleus, the time to peak and the time constant of relaxation were not different from Con values (Table 1). Additionally, the time to peak and the time constant of relaxation were similar in HU, CNTF-HU, and Con EDL (Fig. 3, Table 1).

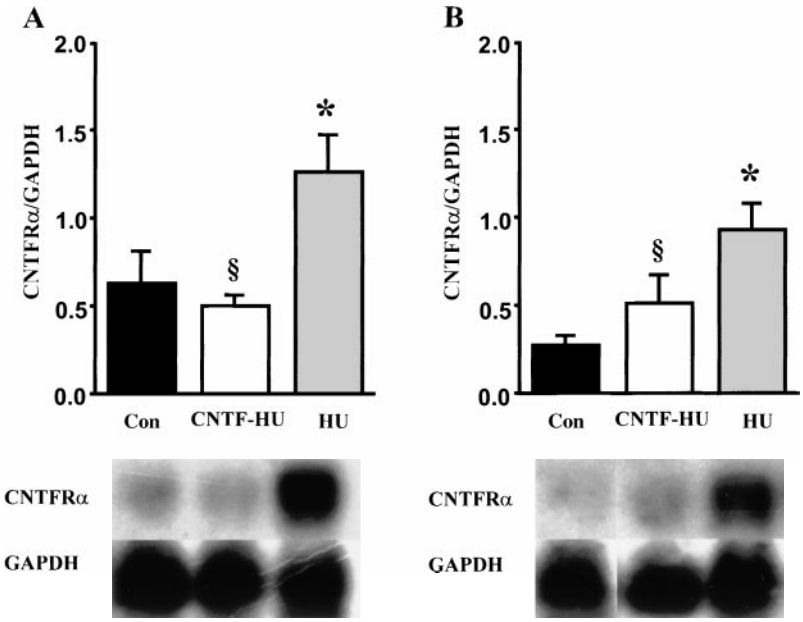

Fig. 2. *Top*: ratio of CNTF receptor-α (CNTFR-α) to glyceraldehyde-3-phosphate dehydrogense (GAPDH) in soleus (*A*) and EDL (*B*) muscles. Values are means ± SE; $n = 5$ for EDL and soleus in each group. Significantly different from *Con muscle and §HU muscle, $P < 0.05$. *Bottom*: Northern blots hybridized with CNTFR-α or GAPDH were scanned, and ratio of the peak surfaces was determined for each muscle. Representative Northern signals (CNTFR-α and GAPDH signals) for each muscle were placed under its corresponding bar (*top*).

K contractures. After 21 days of tail suspension, the 146 mM K tension contracture of HU soleus was significantly reduced by ~40% compared with that of the Con muscle (Fig. 4, Table 2). Tension generated by 146 mM K contracture was significantly larger in CNTF-HU soleus than in HU muscle (Fig. 4, Table 2). In contrast, no significant difference in force generated by 146 mM K contracture could be observed between CNTF-treated unweighted, CNTF-treated normal loaded, and Con soleus (Fig. 4, Table 2). In EDL, the tension developed by 146 mM K contracture did not significantly differ in HU, CNTF-HU, and Con muscles (Table 2). On the other hand, for soleus and EDL, the 146 mM K contracture-to-Triton contracture tension ratio was similar among CNTF-HU, HU, and Con muscles (Table 2).

The time to peak and the time constant of relaxation of 146 mM K contractures were not different among HU, CNTF-HU, and Con soleus (Fig. 4, Table 2). On the other hand, kinetic parameters of 146 mM K contractures measured in soleus muscles did not differ from those of EDL muscles (Table 2). Moreover, the time to peak and the time constant of relaxation of 146 mM K contractures were not different in contralateral unweighted, CNTF-treated unloaded, and normal EDL (Fig. 4, Table 2).

After 21 days of unloading strength, the recovery of test 146 mM K contracture was slower in contralateral unloaded soleus than in normal muscle (Fig. 5). The R_{50}

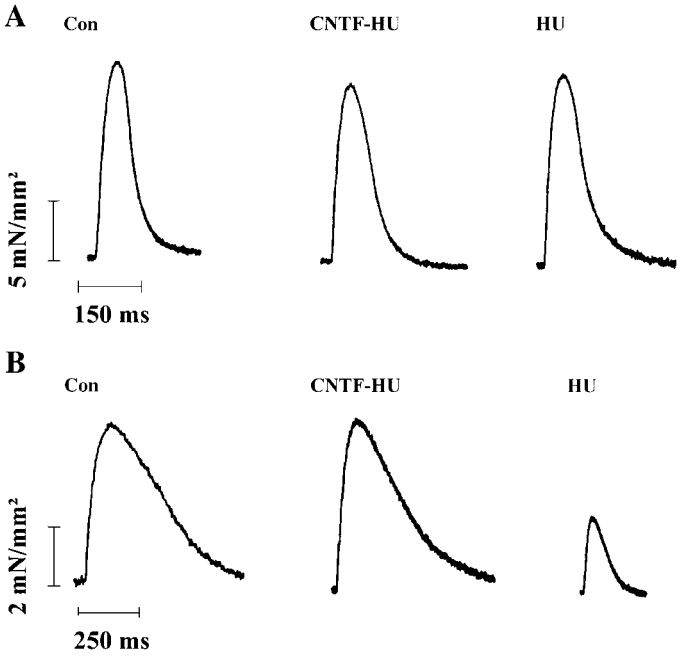

Fig. 3. Effect of CNTF treatment and suspension on isometric twitch of soleus and EDL muscles. Shown are recording of twitch from EDL (*A*) and soleus (*B*) muscles. Experiments were conducted at 19–21°C.

Table 1. *Effect of CNTF treatment and unweighting on twich characteristics of soleus and EDL muscles*

	TT, mN/mm²	TPT, ms	CT, ms
Soleus			
Con	5.4 ± 0.6	116 ± 10	229 ± 22
CNTF-Con	4.4 ± 1.2	122 ± 16	239 ± 32
CNTF-HU	5.7 ± 0.9	82 ± 7	167 ± 11
HU	2.5 ± 0.5*†	61 ± 3*†	106 ± 11*†
EDL			
Con	16.5 ± 4.1	47 ± 5	68 ± 8
CNTF-HU	12.3 ± 3.1	46 ± 3	62 ± 9
HU	12.2 ± 2.3	48 ± 2	63 ± 7

Values are means ± SE. Eight rats were studied per group; at least two intact fibers were used by muscle. EDL, extensor digitorum longus; TT, twitch tension; TPT, time to peak tension; CT, constant of relaxation; Con, control muscle; CNTF-Con, ciliary neurotrophic factor (CNTF)-treated muscle from normal loaded rats; CNTF-hindlimb unweighted (HU), CNTF-treated muscle from unloaded rats; HU, contralateral muscle from unloaded rats. *Significantly different from Con muscle ($P < 0.05$); †significantly different from CNTF-HU muscle ($P < 0.05$).

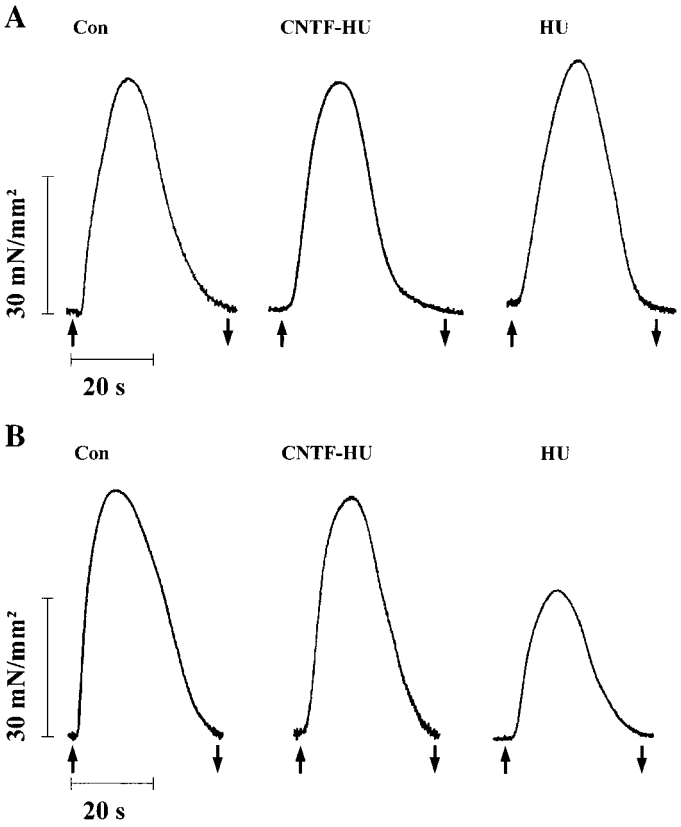

Fig. 4. Effect of CNTF treatment and suspension on 146 mM K contractures of soleus and EDL muscle. Shown are recording of 146 mM K contracture from EDL (*A*) and soleus (*B*) muscles. Space between up and down arrows corresponds to duration of challenge in depolarizing solution. Experiments were conducted at 19–21°C.

values were 75 ± 10 s (n = 8) and 48 ± 6 s (n = 8) for contralateral and normal soleus, respectively ($P <$ 0.05). Conversely, the recovery tension of the test contracture in CNTF-treated unloaded soleus was significantly accelerated compared with that in normal muscle, with an R_{50} value of 33 ± 3 s (n = 8, $P <$ 0.05) (Fig. 5). On the other hand, the R_{50} value calculated for CNTF-HU (50 ± 6 s, n = 8) was not changed compared

Table 2. *Effect of CNTF treatment and unweighting on 146 mM K contracture characteristics of soleus and EDL muscles*

	KT, mN/mm²	TPT, s	CT, s	KT/Triton T, %
Soleus				
Con	54 ± 7	9.5 ± 0.8	11.2 ± 1.1	86 ± 8
CNTF-Con	58 ± 9	9.7 ± 0.9	9.0 ± 0.8	88 ± 5
CNTF-HU	52 ± 4	9.0 ± 0.6	8.9 ± 0.8	85 ± 2
HU	36 ± 4*†	10.5 ± 0.7	9.9 ± 0.9	85 ± 3
EDL				
Con	43 ± 4	10.5 ± 1.0	11.5 ± 1.8	83 ± 3
CNTF-HU	45 ± 5	11.2 ± 1.3	8.7 ± 1.1	86 ± 2
HU	46 ± 7	11.2 ± 1.2	11.6 ± 1.3	81 ± 5

Values are means ± SE. Eight rats were studied per group; at least two intact fibers were used by muscle. KT, 146 mM K contracture tension; KT/Triton T, 146 mM K contracture tension-to-Triton tension ratio. *Significantly different from Con muscle ($P <$ 0.05); †significantly different from CNTF-HU muscle ($P <$ 0.05).

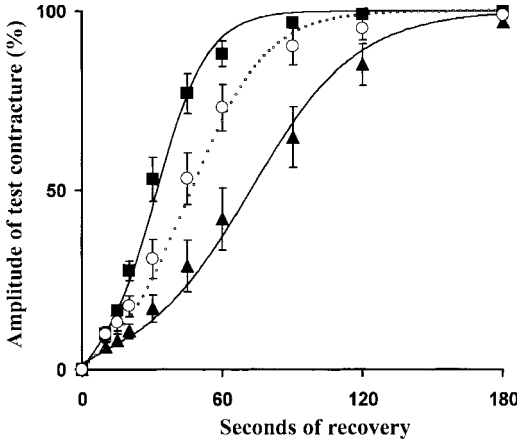

Fig. 5. Time course of repriming of 146 mM K contracture in soleus muscles. ■, CNTF-treated unloaded; ○, Con; and ▲, contralateral unloaded soleus. Each point shows average amplitude of test contracture recorded after different periods of recovery (15–180 s) in normal Ringer solution and is expressed as percentage of conditioning contracture. Bars are SE; n = 8 rats per point. Curves were obtained by fitting Boltzmann equations to mean data. Experiments were conducted at 19–21°C.

with the Con value ($P >$ 0.05). For contralateral unweighted, CNTF-treated unloaded, and normal EDL, R_{50} values did not differ (49 ± 10 s, n = 8; 44 ± 9 s, n = 8; and 42 ± 8 s, n = 8, respectively; $P >$ 0.05).

DISCUSSION

The aim of the present study was to investigate the effects of exogenously provided CNTF on suspension-induced atrophy and functional changes occurring in rat skeletal muscle.

The body weight gains of normal weight-bearing CNTF-treated and nontreated rats were similar. Moreover, the body weight gain of suspended CNTF-treated animals was altered but remained in the range of precedent studies (2, 30). These data indicate that the body weight loss observed in CNTF-treated suspended animals is mainly related to microgravity conditions.

In contralateral unloaded skeletal muscles, the muscle-to-body weight ratio and the total muscle protein content were similar to those previously measured in suspended, nontreated rats (15, 29, 33). Additionally, the muscle-to-body weight ratio of CNTF-Con soleus was not different compared with that of Con muscle. These data indicate that the changes in contralateral muscles were mainly due to unweighting-induced atrophy.

By contrast, muscle-to-body weight ratio and total protein content were significantly larger in CNTF-treated unweighted soleus than in contralateral muscle. These observations suggest that weight and total protein loss induced by suspension in the soleus were in part compensated by CNTF treatment. On the other hand, no significant difference was found among CNTF-treated, contralateral, and normal EDL.

CNTF was delivered with the use of an osmotic pump implanted under the left hindlimb skin of the unloaded animal. Our results show that CNTF had preventive effects on the atrophy that occurred in CNTF-HU

soleus but not in contralateral soleus. It could then be proposed that the CNTF-delivering technique used in this study allows a locally restricted action of the cytokine. This is in accordance with previous studies. Particularly, it has been shown that injected CNTF is rapidly removed from the circulation by the liver and then presents a short plasma half-life (8). Therefore, the present observations allow the estimation of CNTF effects on suspension-induced muscular alterations by comparing muscles of the hindlimb, where the cytokine is delivered, with those of the other leg.

It is known that the soleus muscle unloading results in mechanical property alterations. Isometric twitch and maximal tensions are reduced in unloaded soleus (32), and a shift in the isometric twitch kinetic characteristics toward those of fast-twitch muscles, such as EDL, is observed (11, 16). However, no significant difference has been found between contractile characteristics of normal and unloaded EDL (11). According to these results, the contractile characteristics presently obtained were not significantly altered in contralateral EDL compared with muscle from normal loaded age-matched rats. Additionally, CNTF treatment did not alter contractile properties of normal loaded soleus. Therefore, the specific contractile alterations, which occurred in contralateral soleus, suggest that these functional changes be induced by the 21-day suspension period.

The K contracture decay and conditioning contracture tension repriming have been related to the inactivation and the repriming of voltage sensor implied in the excitation-contraction coupling (ECC) mechanism (ECC voltage sensor) of skeletal muscle (9). The kinetic characteristics of 146 mM K contracture were similar in EDL and soleus, independent of CNTF administration and suspension. Therefore, ECC voltage-sensor inactivation seems to be independent of muscle-type specificity, suspension, and CNTF treatment. Conversely, the R_{50} value increased in HU soleus, suggesting that ECC voltage-sensor repriming is lowered by suspension. Additionally, as R_{50} was smaller in CNTF-HU soleus than in Con, CNTF treatment appears to be able to overcompensate for the suspension-induced repriming lowering of the ECC voltage sensor. The R_{50} values of HU, CNTF-HU, and Con EDL were not different. These data indicate that the repriming of the ECC voltage sensor is altered by suspension and CNTF administration in soleus muscle in a specific manner.

In the CNTF-treated unloaded and normal soleus, isometric twitch tension and strength developed by 146 mM K contracture were not significantly different. Moreover, our results show that, independent of CNTF administration, suspension, and muscle type, 146 mM K still represent ~85% of maximal tension estimated by Triton contracture tension measurement. These data indicate that CNTF seems to be able to prevent the suspension-induced tension decrease in soleus muscle. On the other hand, no change was found in twitch characteristics between CNTF-treated unweighted and normal soleus. Conversely, in CNTF-treated unloaded soleus, the R_{50} was smaller than that in normal soleus. These results indicate that CNTF treatment during suspension is able to reduce functional changes, which occurred in unloaded soleus.

The fact that CNTF treatment reduces unweighting-induced functional changes solely in the hindlimb where the osmotic pump is implanted reinforces the idea that CNTF acts in a local manner. Moreover, as it was shown in unloaded soleus (15), CNTFR-α mRNA expression was found to be larger in HU than in Con soleus, but no difference was measured between CNTF-treated and Con muscles. DiStefano and collaborators (7) have shown that, in normal soleus, frequent CNTF administration results in a profound downregulation of CNTFR-α mRNA. These authors also observed a less pronounced downregulation effect of CNTF administration on CNTFR-α protein. Moreover, they found that CNTF biological signals continue to be recognized and interpreted by the cell (7). Therefore, the CNTFR-α mRNA expression measured in CNTF-treated soleus after 21 days of suspension appears to be the combined effect of a downregulation, due to continuous CNTF delivery, and an upregulation, induced by suspension. On the other hand, CNTFR-α mRNA expression in unloaded contralateral soleus is not reduced and could be accounted for by the short half-life of the CNTF.

In contrast to soleus muscle, no significant change in atrophy and contractile property parameters was observed between CNTF-treated and contralateral unloaded EDL. However, CNTFR-α mRNA expression in contralateral EDL, as in contralateral soleus, was significantly larger than that in normal and CNTF-treated muscles. This result suggests that CNTFR-α mRNA upregulation is not muscle type specific and results from suspension rather than atrophy alteration. Unloaded-induced atrophy and functional changes and CNTF preventive effects were more marked in soleus than in EDL. Furthermore, CNTF treatment did not affect the soleus of normal weight-bearing rats. Therefore, it could be proposed that the action of CNTF is related to the magnitude of the alteration, which mainly affects slow-twitch muscle under unweighting conditions.

The muscle-to-body weight ratio is significantly larger in CNTF-treated unweighted soleus than in the contralateral muscle. It has been demonstrated that a part of the microgravity-induced loss of soleus protein can be attributed to the loss of myofibrillar proteins. After 28 days of unweighting, 80% of the myofibrillar proteins in the soleus muscle was lost (35). This loss was larger in contractile proteins than in other proteins, and it was typical of slow-twitch muscles. Moreover, it has been shown that sarcoplasmic reticulum calcium pump and dihydropyridine receptor gene regulations were affected by suspension in soleus (22, 31). CNTF was reported to be able to trigger gene regulations through its receptor components glycoprotein-130 and leukemia inhibitory factor receptor (19). Therefore, a preferential action of the CNTF on the expression of proteins related to muscular function could explain the more pronounced effects of the cytokine on microgravity-

induced functional changes than on atrophy. The lack of CNTF treatment effect on unloading-induced atrophy and the absence of significant functional alteration in CNTF-HU EDL and CNTF-Con soleus support this idea.

It has been shown that CNTF treatment prevented, only in part, the denervation-induced atrophy and functional change (17). Alterations and atrophy that occurred in denervated skeletal muscle are a composite of disuse (as met in unweighted conditions) and the loss of trophic substances. It was then proposed that CNTF treatment reduced only the trophic component. Our results clearly showed that CNTF could have preventive effects on innervated unweighted soleus but not on normal loaded muscle.

The motoneuron electrical activity is crucial for regulating muscle fiber properties (16). For instance, electrical stimulation of denervated slow-twitch soleus muscle with an impulse pattern resembling that of a nerve innervating a fast-twitch muscle results in several contractile parameters changing in the fast direction (23). During suspension, a reduced and a changed pattern of electromyogram activity were observed in soleus and plantaris muscles (2). These electromyogram alterations may be involved in the mechanism by which muscle senses the absence of gravity with an adaptive atrophy. On the other hand, CNTF was primarily characterized by its ability to sustain the survival of motoneurons in vitro and in vivo. The present results could then be related to a possible effect of the CNTF on the electrical neuron activity and/or directly on the muscle fiber.

The major findings of our study are that CNTF is able to reduce contractile alterations, which occurred in unloaded soleus. On the other hand, the regulation of CNTF signal transduction machinery seems to be similarly altered in fast- and slow-twitch muscles. These results suggest that exogenously provided CNTF could regulate muscular function rather than skeletal muscle-type specificity. However, further studies are needed to determine in which way CNTF is able to reduce nonweight-bearing-induced functional changes.

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