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SKELETAL MUSCLE

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mRNA expression of fibroblast growth factors and hepatocyte growth factor in rat plantaris muscle following denervation and compensatory overload

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Abstract We addressed the question of whether hypertrophy induced by compensatory overload differs according to innervation status, and how fibroblast growth factors (FGF) and hepatocyte growth factor (HGF) mRNAs are expressed in the rat plantaris muscle during overload (OL) and/or denervation. Male Wistar rats were divided into four groups (Normal-Cont, Normal-OL, Denervated-Cont, and Denervated-OL). according to the plantaris denervation and/or overload. Three weeks later, plantaris weight in Denervated-Cont and Denervated-OL was significantly lower than in the Normal-Cont. The muscle weights in the Normal-OL were higher than in the Normal-Cont. The muscle weights in the Denervated-OL were higher than in the Denervated-Cont. Three days after the treatment, FGF-2, FGF-6, FGF-7 and HGF mRNAs in the Normal-OL were significantly higher than those in the Normal-Cont. FGF-2, FGF-6, FGF-7 and HGF mRNAs in the Denervated-OL were also significantly higher after 3 days than those in the Denervated-Cont. After 7 days, FGF-2, FGF-5, FGF-6, FGF-7 and HGF mRNAs were significantly higher in the Normal-OL than those in the Normal-Cont. At 21 days, FGF-1, FGF-6 and HGF mRNA levels were significantly increased. In the Denervated-OL, FGF-2, FGF-7 and HGF mRNAs at 7 days, and FGF-2 mRNA at 21 days were significantly higher than those in the Denervated-Cont. FGF-2 and FGF-6 mRNA levels decreased significantly following denervation; however, FGF-1, FGF-5, FGF-7 and HGF mRNA levels increased and maintained this increase for the 21-days treatment period. Muscle hypertrophy was thus induced by compensatory overload irrespective of innervation status, possibly in association with certain FGFs and HGF. The differential mRNA expression patterns of FGFs and HGF observed following compensatory overload and/or denervation suggest distinct roles for individual FGFs and HGF in muscle hypertrophy and/or atrophy.

Keywords FGF · HGF · Myosin heavy chain isoforms · Overload · Denervation

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Introduction

Mammalian skeletal muscles undergo hypertrophy following stretch or compensatory overload induced by removal of synergists [40, 46]. Satellite cells, the normally inactive myogenic nuclei that participate in developmental and regenerative growth of skeletal muscle [35], are known to play an important role in hypertrophy. In response to stretch or compensatory overload, satellite cells are activated to enter the mitotic cycle and proliferate, and activated satellite cells fuse into preexisting fibres or form new fibres [35]. Inactivation of satellite cells by gamma irradiation prevents muscle hypertrophy following compensatory overload [26, 29].

The most prominent feature of denervated skeletal muscle is rapid atrophy of muscle fibres [10, 24, 25]. Short-term denervation induces satellite cells to enter the mitotic cycle [20, 21, 23], whereas long-term denervation decreases the number of both myonuclei and satellite cells [6, 28, 33, 42]. Satellite cells isolated from denervated muscle for 1 week or longer are resistant to recruitment into the mitotic cycle [17]. Thus, it is well established that the behaviour of satellite cells is influenced by muscle denervation, although little is known about the factors influencing such behaviour.

Fibroblast growth factors (FGFs) stimulate proliferation and inhibit differentiation in myogenic cell lines [1, 15, 27], and FGF-1, FGF-2, FGF-5, FGF-6, FGF-7 and hepatocyte growth factor (HGF) are expressed in rat satellite cells isolated from myofibres [16]. FGFs are considered to be powerful regulators of skeletal muscle in vitro and may be associated with muscle hypertrophy and atrophy in vivo. However, the expression patterns of FGFs and HGF during overload-induced hypertrophy in mammalian skeletal muscle are not well understood, and even less is known about these patterns in denervated skeletal muscle.

In cultured myofibres, continuous rhythmic stretching results in increased protein synthesis and prevents the atrophy that is noted in non-stimulated muscle cells [41]. Low-frequency electrical stimulation transforms fast muscle fibres into slow muscle fibres in denervated muscle, whilst high-frequency stimulation produces a change in the reverse direction [12]. These results suggest that changes in fibre sizes and types in skeletal muscle are important to muscle activity itself. However, there is no evidence that passive stretching independent of neural input in vivo causes muscle hypertrophy. We have developed a model of passive stretching of the plantaris muscle independent of neural input by combining ablation of the synergists with surgical denervation of the tibial nerve, which leaves intact the neural input and contractile activity of the antagonists. Using this model, we determined whether hypertrophy induced by compensatory overload differs according to innervation status and the mRNA expression patterns of FGFs and HGF during overload and/or denervation.

Materials and methods

Animal care and surgical procedures

Male Wistar rats aged 10 weeks and weighing 200–230 g were used. All animals were housed in cages at a constant room temperature (22 ±2 °C) and fed ad libitum. All surgical procedures were performed under anaesthesia. All experiments and procedures conformed to the Guideline for the Care and Use of Laboratory Animals of the Health Sciences University of Hokkaido. Experiments were initiated by the unilateral removal of the gastrocnemius and soleus muscles [45], and sham operations were carried out on the contralateral leg, which served as the control (Cont). Also, the animals were allocated randomly into innervated and denervated groups. Rat calf muscles (plantaris, soleus and gastrocnemius) are innervated by the tibial nerve whilst the antagonists (tibialis anterior and extensor digitorum longus muscles) are innervated by the common fibular nerve. Denervation was performed by cutting the tibial nerve bilaterally. For the denervated control leg (Denervated-Cont), the common fibular nerve was also cut. For the denervated overloaded leg (Denervated-OL), the common fibular nerve was left intact. Consequently, the plantaris muscle in the Denervated-OL underwent passive stretch via the contractile activities of its antagonists. In the Denervated-Cont, the passive stretch of the plantaris muscle was prevented by simultaneous denervation of its antagonists. To prevent reinnervation, 5-mm segments of the nerves were removed and the proximal stumps ligated. The overloaded and control legs in the innervated group served as Normal-OL and Normal-Cont, respectively. This study thus comprised four groups: Normal-Cont, Normal-OL, Denervated-Cont, and Denervated-OL, according to denervation and/or overload status. The animals were sacrificed 3, 7 or 21 days after treatment. The plantaris muscle was dissected quickly, freed of any fat and connective tissue, weighed, frozen in liquid nitrogen and stored at −80 °C.

Analysis of myosin heavy chain (MHC) isoforms

Six muscles of each group 21 days after compensatory overload and/or denervation were used for the analysis of MHC isoforms by SDS-PAGE according to [30]. Small muscle pieces were homogenized in 40 vol 5 M urea, 2 M thiourea, 10 mM sodium pyrophosphate and 0.1% 2-mercaptoethanol using a glass homogenizer. The homogenates were diluted 75-fold in a solution composed of 62.5 mM TRIS-HCl (pH 6.8), 2% SDS, 10% glycerol, 5% 2-mercaptoethanol and 0.02% bromophenol blue and loaded onto the gel. The stacking gel comprised 3.5% acrylamide, 0.07% bis-acrylamide, 70 mM TRIS-HCl (pH 6.8), 0.4% SDS, 0.1% *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) and 0.1% ammonium persulphate. The separating gel was 7.5% acrylamide, 0.2% bis-acrylamide, 30% glycerol, 200 mM TRIS/100 mM glycine (pH 8.6), 0.4% SDS, 0.1% TEMED and 0.06% ammonium persulphate. SDS-PAGE was carried out at a constant voltage of 200 V (4 °C) for 36 h. After SDS-PAGE, gels were silver-stained using a Wako Silver Stain Kit (Wako, Tokyo, Japan). The percentage distribution of MHC isoforms was estimated using NIH Image 1.54.

Analysis of gene expression by RT-PCR

Six muscles of each group 3, 7 or 21 days after compensatory overload and/or denervation were used for the analysis of the mRNA expression of FGFs and HGF. Total RNA was extracted by the guanidinium isothiocyanate-phenol-chloroform method [5]. RNA concentrations were determined spectrophotometrically at 260 nm and the integrity of the RNAs was determined by gel electrophoresis. Total RNA (1 μg) was used to synthesize first-strand cDNA in reverse transcriptase (RT) buffer containing 1×PCR buffer (Applied Biosystems, Tokyo, Japan), 5 mM $MgCl_2$, 1 mM deoxynucleic acid triphosphates (dNTPs), 50 ng/μl oligo(dT) primer (Novagen, Darmstadt, Germany), 1 U/μl ribonuclease inhibitor (Takara, Tokyo, Japan), and 0.25 U/μl avian myeloblastosis virus (AMV)-reverse transcriptase XL (Takara) in a total volume of 20 μl. For amplification, a PCR master mixture containing 1×PCR buffer (Applied Biosystems), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.025 U/μl Taq polymerase (Ampli Taq Gold, Applied Biosystems) and 0.4 μM of each sense and antisense primer specific for FGF-1, FGF-2, FGF-5, FGF-6, FGF-7, FGF-8 and hepatocyte growth factor (HGF) was prepared just before use. From this mixture, a 29-μl aliquot was removed and added to 1 μl cDNA and then placed in a temperature-controlled system (PCR Thermal Cycler SP, Takara). The PCR cycling program consisted of denaturation at 94 °C for 30 s, annealing at various temperatures (53.5–58 °C) for 30 s and elongation at 72 °C for 90 s. The PCR products were electrophoresed through 2% agarose gels, stained with ethidium bromide, photographed and quantified using NIH Image 1.54.

Specific primers used are summarized in Table 1. Each PCR primer was designed using OLIGO software (Medprobe) or published sequences [16] and the oligonucleotides were purchased from Hokkaido System Science (Sapporo, Japan). The RT-PCR products were sequenced by a double-strand sequencing service (Hokkaido System Science) to confirm the corresponding rat sequences. In a preliminary experiment, each cDNA was amplified for various cycle numbers (cycles 33–37 for FGF-1, cycles 29–33 for FGF-2, cycles 29–36 for FGF-5, cycles 34–38 for FGF-6, cycles 26–30 for FGF-7, cycles 30-36 for FGF-8 and cycles 32–36 for HGF) to establish a suitable PCR amplification ($cDNA_n$) and amplification efficiency (R) of the unsaturated logarithmic phase. R remained constant during the unsaturated logarithmic phase and did not differ between groups. The estimation was repeated using all the primers, and identical results were obtained. The cDNA value at cycle 0 ($cDNA_0$) was calculated from the equation $\log cDNA_n = \log cDNA_0 + n \cdot \log(1+R)$, where n is the cycle of amplification [11, 14]. As the $cDNA_0$ is proportional to the quantity of starting mRNA in the sample [11, 14], this allowed quantitative comparisons of the PCR product between groups. The $cDNA_0$ value for each PCR product is shown relative to the value in Normal-Cont group (=1.0). In our preliminary experiments, FGF-4 was expressed only in rat embryonic calf muscle and not in adult muscle, whilst FGF-3 was not detectable in embryonic or adult calf muscles (A. Yamaguchi, unpublished observations). Consequently, FGF-3 and FGF-4 were not analysed in this study.

Statistics

Standard procedures were employed to calculate the means and SD. One-way ANOVA was used for the comparison between groups. Differences between the means were regarded as significant at $P<0.05$.

Results

Figure 1 shows the weights of the plantaris muscle in each group 21 days after compensatory overload and/or denervation. The muscle weight in the Normal-OL group was significantly higher than in the Normal-Cont group. The muscle weights in the Denervated-Cont and Denervated-OL groups were significantly lower than in the Normal-Cont group. The muscle weight in the Denervated-OL group was significantly higher than in the Denervated-Cont group.

MHC isoforms in the plantaris muscle 21 days after compensatory overload and/or denervation are shown in Table 2. The Normal-OL group possessed a higher percentage of the type-I MHC isoform than the Normal-Cont group. The Denervated-OL group also possessed a higher percentage of type-I MHC than the Denervated-Cont group. The Denervated-Cont and Denervated-OL groups possessed higher percentages of types-I and -IIa MHC isoforms and a smaller percentage of the type-IIb MHC isoform than the Normal-Cont group.

Figure 2 shows FGF-1 mRNA expression in the plantaris muscle 3, 7 and 21 days after compensatory overload and/or denervation. FGF-1 mRNA expression 3 days after treatment did not differ among groups. FGF-1 mRNA levels in the Normal-OL group 21 days after compensatory overload were significantly higher than

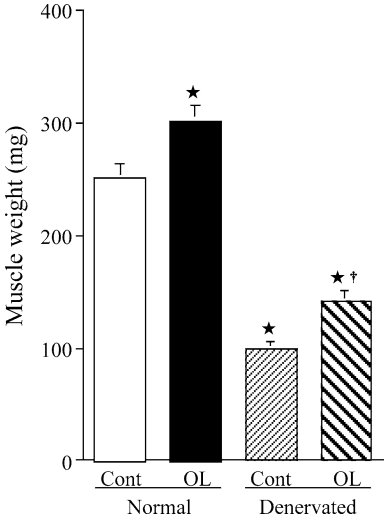

Fig. 1 The weights of rat plantaris muscles 21 days after compensatory overload (*OL*) and/or denervation. *$P<0.05$ vs. non-denervated, non-overloaded muscle (*Normal-Cont*); †$P<0.05$ vs. the denervated non-overloaded muscle (*Denervated-Cont*)

Table 1 Primers for RT-PCR. The primers were derived from the following GeneBank accession numbers: FGF-1: X14232; FGF-2: M22427; FGF-5: D64085; FGF-6: AB079674; FGF-7: X56551; FGF-8: AB079113; HGF: D90102

Gene	Sequence (5′–3′)	Position (5′–3′)	Size (bp)	Suitable cycles
FG-1	GACAGTGGAACGCAGGTGG	163–181	556	37
	GGGACGGGGAGAAACAAGA	718–700		
FG-2	GCAGCATCACTTGCGTTCC	543–561	437	32
	TGGAAGAAACAGTATGGCCTTCTG	979–956		
FGF-5	GTCTCTCAGGGGATTGTAGGAATACG	370–395	134	34
	GTGAAGGAAAGTTCCGGTTGC	983–663		
FGF-6	GGCTCTCGTCTTCTTAGGC	69–87	452	37
	CGGACTCGTAGGCGTTGTA	520–502		
FGF-7	ATCCTGCCGACTCCGCTCTA	133–152	487	29
	CCTTTTGATTTAAGGCCACGAACA	619–596		
FGF-8	AGCAACGGCAAAGGCAAGG	325–343	240	34
	GAAGGGCGGGTAGTTGAGG	564–546		
HGF	TTGCCCTATTTCCCGTTGT	1534–1552	565	35
	CTTGCCTTGATGGTGGTGA	2098–2080		

Table 2 Myosin heavy chain isoforms in the rat plantaris muscle 21 days after compensatory overload (*OL*) and denervation. Means ±SD (*Normal* non-denervated, *Cont* non-overloaded)

	type I	type IIa	type IId	type IIb
Normal-Cont	3.7±1.1	29.6±0.6	39.3±5.6	27.5±4.9
Normal-OL	7.3±0.9*	31.3±1.5	37.1±1.5	24.2±2.7
Denervated-Cont	5.8±1.3*	34.1±1.6*	39.0±2.0	21.2±2.9*
Denervated-OL	7.9±2.4*,†	32.4±2.2*	39.0±2.6	20.8±3.4*

*$P<0.05$ vs. Normal-Cont
†$P<0.05$ vs. Denervated-Cont

those in the Normal-Cont group. After 7 and 21 days, FGF-1 mRNAs in the Denervated-Cont and Denervated-OL groups were significantly higher than those in the Normal-Cont group.

FGF-2 mRNA levels in the Normal-OL group 3 and 7 days after compensatory overload were significantly higher than those in the Normal-Cont group. FGF-2 mRNA levels in the Denervated-OL group after 3, 7 and 21 days were significantly higher than those in the Denervated-Cont group. FGF-2 mRNA levels in the Denervated-Cont group after 3 and 21 days were significantly lower than those in the Normal-Cont group (Fig. 3).

FGF-5 mRNA levels in the Normal-Cont group 3 and 21 days after compensatory overload did not differ from those in the Normal-OL group, however, FGF-5 mRNA level in the Normal-Cont group after 7 days was significantly higher than those in the Normal-Cont

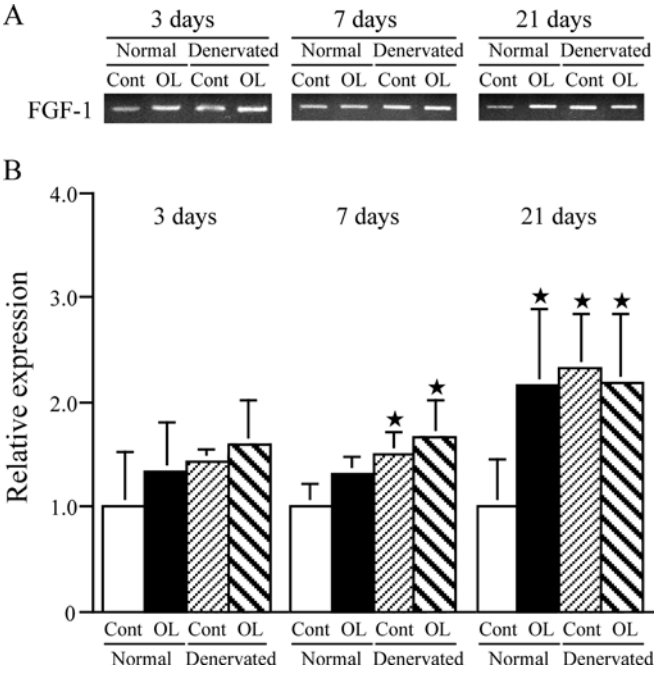

Fig. 2A, B Fibroblast growth factor-1 (*FGF-1*) mRNA expression in rat plantaris muscles 3, 7 and 21 days after compensatory overload and/or denervation. **A** Ethidium-stained products; **B** FGF-1 mRNA expression relative to that in the Normal-Cont group (=1.0). *$P<0.05$ vs. Normal-Cont

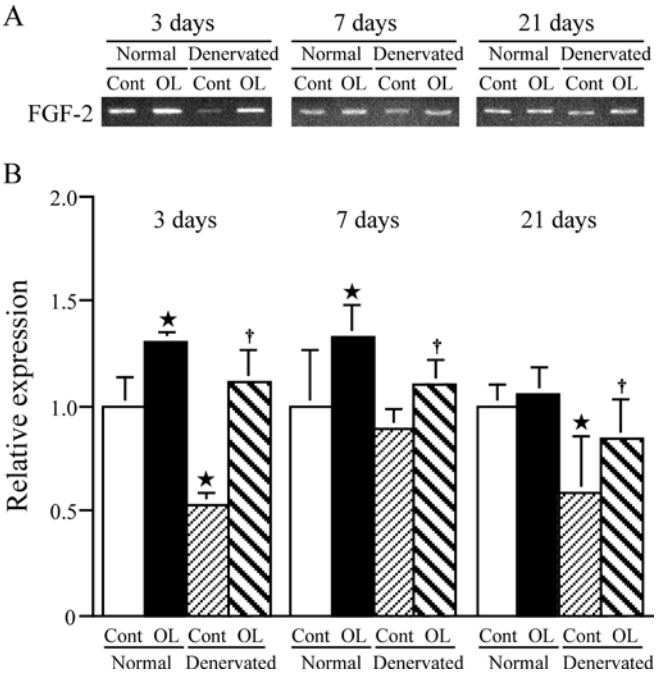

Fig. 3A, B FGF-2 mRNA expression in the plantaris muscle 3, 7 and 21 days after compensatory overload and/or denervation. **A** Ethidium-stained products; **B** FGF-2 mRNA expression relative to that in the Normal-Cont group (=1.0). *$P<0.05$ vs. Normal-Cont. †$P<0.05$ vs. Denervated-Cont

group. FGF-5 mRNA levels in the Denervated-Cont group after 3, 7 and 21 days did not differ from those in the Denervated-OL group. FGF-5 mRNA levels in the Denervated-Cont and Denervated-OL groups after 3, 7 and 21 days were significantly higher than those in the Normal-Cont group (Fig. 4).

FGF-6 mRNA levels in the Normal-OL group 3, 7 and 21 days after compensatory overload were significantly higher than those in the Normal-Cont group. Also, the FGF-6 mRNA level in the Denervated-OL group after 3 days was significantly higher than that in the Denervated-Cont group. FGF-6 mRNA levels in the Denervated-Cont group after 3, 7 and 21 days were significantly lower than those in the Normal-Cont group. FGF-6 mRNA levels in the Denervated-OL group 7 and 21 days after the treatments were significantly lower than those in the Normal-Cont group (Fig. 5).

FGF-7 mRNA levels in the Normal-OL group 3, 7 and 21 days after compensatory overload were significantly higher than those in the Normal-Cont group. Also, FGF-7 mRNA levels in the Denervated-OL group after 3 and 7 days were significantly higher than those in the Denervated-Cont group. FGF-7 mRNA levels in the Denervated-Cont and Denervated-OL groups after 3, 7 and 21 days were significantly higher than those in the Normal-Cont group (Fig. 6).

FGF-8 mRNA expression in the plantaris muscle 3, 7 and 21 days after compensatory overload and/or denervation did not differ among the groups.

HGF mRNA levels in the Normal-OL group 3, 7 and 21 days after compensatory overload were significantly

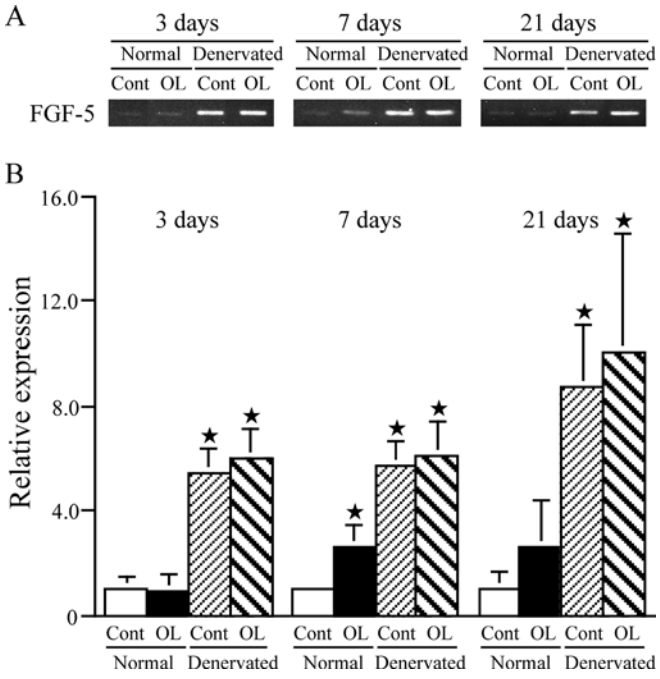

Fig. 4A, B FGF-5 mRNA expression in the plantaris muscle 3, 7 and 21 days after compensatory overload and/or denervation. **A** Ethidium-stained products; **B** FGF-5 mRNA expression relative to that in the Normal-Cont group (=1.0). **P*<0.05 vs. Normal-Cont

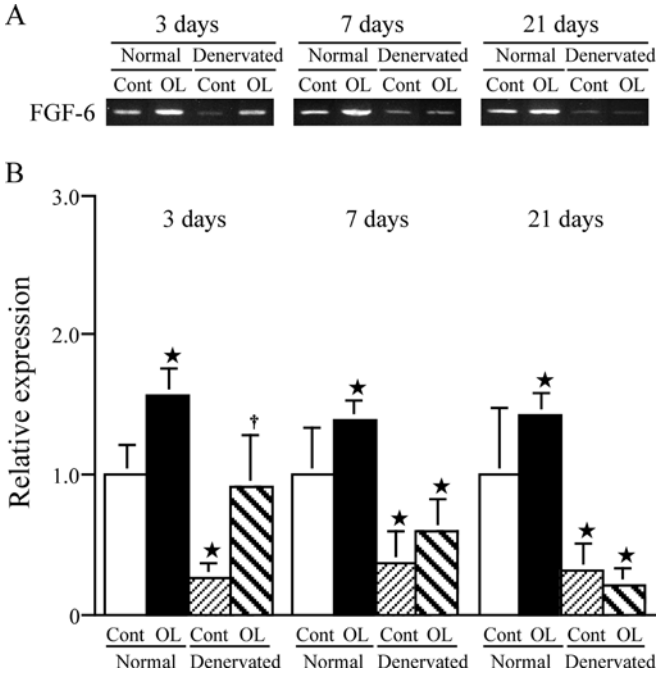

Fig. 5A, B FGF-6 mRNA expression in the plantaris muscle 3, 7 and 21 days after compensatory overload and/or denervation. **A** Ethidium-stained products; **B** FGF-6 mRNA expression relative to that in the Normal-Cont group (=1.0). **P*<0.05 vs. Normal-Cont. †*P*<0.05 vs. Denervated-Cont

higher than those in the Normal-Cont group. Also, HGF mRNA levels in the Denervated-OL group after 3 and 7 days were significantly higher than those in the Denervated-Cont group. HGF mRNA levels in the Denervated-Cont group after 3 and 7 days were significantly higher than those in the Normal-Cont group. HGF mRNA levels in the Denervated-OL group after 3, 7 and 21 days were significantly higher than those in the Normal-Cont group (Fig. 7).

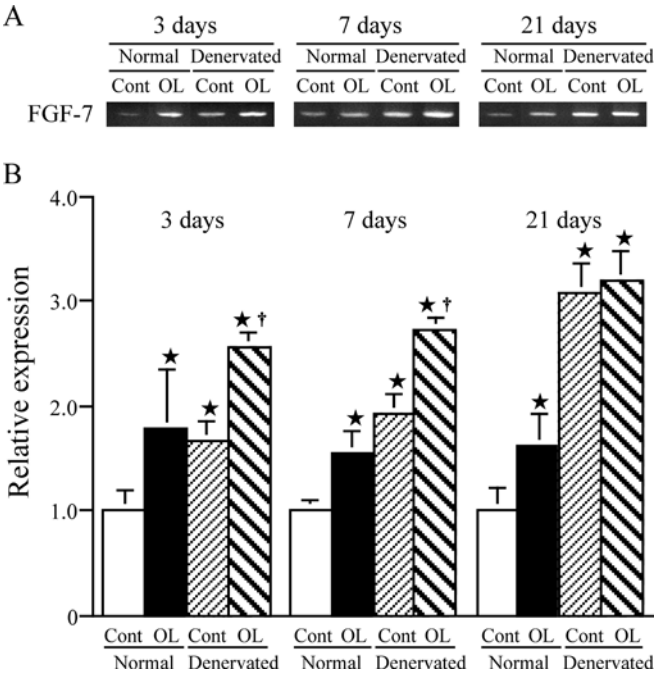

Fig. 6A, B FGF-7 mRNA expression in the plantaris muscle 3, 7 and 21 days after compensatory overload and/or denervation. **A** Ethidium-stained products; **B** FGF-7 mRNA expression relative to that in the Normal-Cont group (=1.0). **P*<0.05 vs. Normal-Cont. †*P*<0.05 vs. Denervated-Cont

Discussion

In this study, the expression of FGF-2, FGF-6, FGF-7 and HGF mRNAs increased shortly after initiating the compensatory overload, but not that of FGF-1, FGF-5 and FGF-8 mRNAs. Denervation reduced the FGF-2 and FGF-6 mRNA levels, increased the FGF-1, FGF-5, FGF-7 and HGF mRNA levels but did not change the FGF-8 mRNA level, in comparison with the innervated plantaris. Adding FGFs and HGF to cultured rat myofibres, FGF-1, FGF-2, FGF-4, FGF-6 and HGF enhance satellite cell proliferation, whereas FGF-5 and FGF-7 do not [16]. Moreover, several, but not all, FGFs are expressed in response to stretch-induced hypertrophy in avian muscle [22]. Thus, even if they belong to the FGF family, the gene expression differs in response to different stimuli. It is likely that individual FGFs do not always play the same role in the morphologic growth or maintenance of skeletal muscle.

In this study, the expression of FGF-2, FGF-6, FGF-7, and HGF mRNAs increased with overload-induced hypertrophy. Although the roles of FGFs and HGF in mammalian skeletal muscle in vivo remain obscure, these observations raise the possibility that overload-induced muscle hypertrophy may be influenced by certain FGFs.

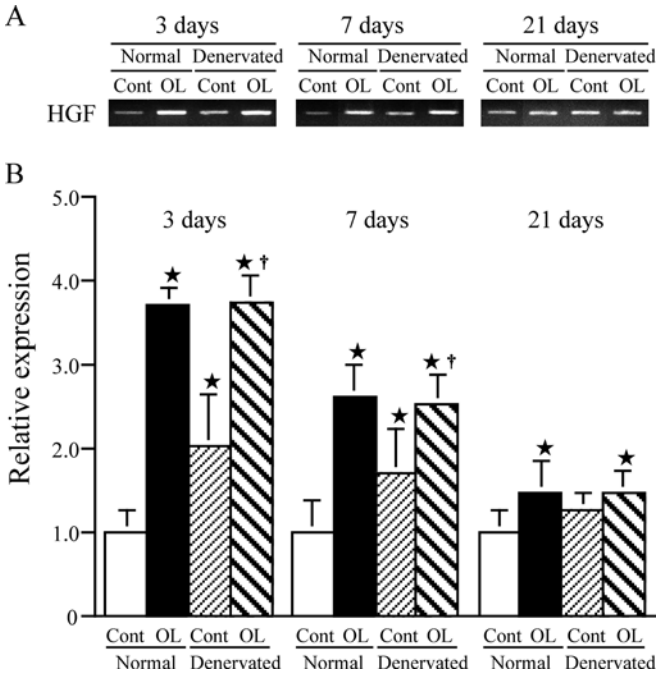

Fig. 7A, B Hepatocyte growth factor (*HGF*) mRNA expression in the plantaris muscle 3, 7 and 21 days after compensatory overload and/or denervation. **A** Ethidium-stained products; **B** HGF mRNA expression relative to that in the Normal-Cont group (=1.0). **P*<0.05 vs. Normal-Cont. †*P*<0.05 vs. Denervated-Cont

FGFs stimulate proliferation and inhibit differentiation in myogenic cell lines [1, 15, 27]. HGF also plays a role in enhancing proliferation in satellite cells and in inhibiting cell differentiation [9]. FGF-2 and FGF-4 are also chemotactic for limb myogenic cells [18, 43] and HGF induces increased chemotaxis of satellite cells [4]. Addition of anti-HGF antibodies to cultured satellite cells eliminates their activation in response to mechanical stretching [39]. In adult muscle, satellite cells are normally quiescent, but become mitotically active in response to various stimuli, including stretching and compensatory overload [35, 36, 44]. The activated satellite cells fuse into preexisting fibres or form new muscle fibres [35]. The inactivation of satellite cells by gamma irradiation prevents muscle hypertrophy following compensatory overload [26, 29]. Because FGF-2 and FGF-6 proteins are expressed in adult rat skeletal muscle [16, 31, 32] and the presence of HGF protein in satellite cells isolated from adult rat muscle has been detected by immunofluorescence [39], the enhanced expression of FGF-2, FGF-6, FGF-7, and HGF during overload in this study may be associated with muscle hypertrophy, possibly by activation of satellite cells.

The FGF-5 mRNA level did not change 3 days after compensatory overload, but was increased at 7 or more days. FGF-2, FGF-6 and HGF mRNA levels were increased at 3 days and maintained this increase during compensatory overload. It is difficult to explain what roles the prolonged expressions of FGFs and HGF mRNAs might have. FGFs and HGF may play an important role in the continuing muscle fibre hypertrophy as well as in the activation of satellite cells. FGFs and HGF may also play a role in immature fibre formation, which is often observed in overloaded muscles [46]. Further studies are needed to elucidate these phenomena

Little is known about the expression pattern of FGFs and HGF in denervated skeletal muscle. The observations in this study indicate that mRNAs of individual FGFs and HGF were expressed very differently patterns following denervation; FGF-2 and FGF-6 mRNA levels were decreased, FGF-1, FGF-5, FGF-7 and HGF mRNA levels increased and FGF-8 mRNA level unchanged following denervation. The functional meaning of these changes following denervation are unknown. FGF-2 and FGF-6 are the most prominent factors in satellite cell activation [1, 8, 16, 27]. As reduced expression of FGF-2 and FGF-6 mRNAs was shown shortly after denervation in this study, these FGFs are most closely associated with denervation atrophy. Little is known about the factors involved in muscle atrophy following denervation. Proliferating cell nuclear antigen-positive and myogenin-positive cells, the proliferative and differentiative compartments of cells, in an isolated cell culture from denervated muscle are decreased after 1 week or more, and denervation for 1 week or longer hinders recruitment into the mitotic cycle [17]. Prolonged denervation decreases the number of myonuclei and satellite cells [6, 28, 33, 42]. These observations suggest that muscle atrophy following denervation may be related to inactivation of myonuclei and satellite cells. Collectively, the reduced expression of FGF-2 and FGF-6 may induce the inactivation of myonuclei and satellite cells and may be related to muscle atrophy following denervation.

In previous studies, satellite cell proliferation has been observed during short-term denervation in vivo and mitotic activation is found in the first few days or weeks after denervation using the [^{3}H]-thymidine incorporation strategy [20, 21, 23]. In this study, FGF-5, FGF-7, and HGF mRNAs increased shortly after denervation. Thus, some FGFs may be associated with the activation of satellite cells after short-term denervation.

The increased expression of FGF-1, FGF-5, and FGF-7 mRNAs was maintained during denervation in this study. Myotubes begin to appear a few weeks after denervation and are present for several months [13, 28, 34]. The myotubes observed during denervation fail to grow and to mature into normal muscle fibres. As two important effects of FGFs observed in in vitro studies are the suppression of cell differentiation and the acceleration of cell proliferation, the enhanced expression of FGFs may also play a role in preventing new fibres from maturing. Ectopic application of FGFs can prevent apoptotic cell death [7] and support cell survival in various organisms [38]. Long-term denervation causes remarkable atrophy of muscle fibres and muscle fibre degeneration, but even though degeneration progressed for 25 months, surviving muscle fibres were found [6]. Thus, FGFs may be related to the survival of fibres in muscle with long-term denervation. Denervation causes a progressive increase in connective tissue [13, 19]. Since FGFs are expressed in connective

tissue as well as in muscle fibres or satellite cells [16], they may play roles not only in the events observed in the denervated muscle fibres, but also in the connective tissue.

An interesting finding of this investigation was that a change in muscle fibre size due to compensatory overload occurred in both denervated and innervated muscle. As the overload in this study resulted from ablation of the plantaris synergists, the hypertrophy of the remaining plantaris muscle resulted from the increased active shortening from various daily activities in compensation for the ablated synergists. However, as the denervated muscle cannot contract spontaneously, the ablation of the denervated synergists could not increase the compensatory active shortening of the plantaris muscle. The only possible effect counteracting atrophy in the denervated plantaris muscle following compensatory overload is increased passive stretching associated with activities of the innervated antagonists. Stretching of the anterior latissimus dorsi muscle in birds reportedly also produces muscle enlargement [2, 3]. In mammalian skeletal muscles, short-term stretching due to plaster cast immobilization in the lengthened position causes an increase of muscle mass [46]. In cultured myofibres, continuous rhythmic stretching increases protein synthesis and prevents the atrophy noted in unstimulated muscle cells [41]. These observations would support the notion that the counteracting effect of the overload on the denervated atrophy is caused by increased passive stretch, independent of neural input. However, the fact that compensatory overload in this study only partly prevented the atrophy in a denervated plantaris muscle should be addressed.

Denervation in this study increased the percentage of types-I and -IIa MHC isoforms at the expense of type-IIb. As we did not estimate the sizes of each fibre type, it is unknown whether the changes in MHC isoforms were dependent on fibre type conversion or selective atrophy of fibre types. Muscle fibre composition following denervation changes slow fibres into fast fibres in slow muscle [10, 12, 37], but not in fast muscle, including the plantaris muscle [10]. Also, type-IIB fibres atrophy more severely than type-I fibres [10, 19, 24, 25]. Taken together, it appears that the decreased percentage of the type IIb MHC isoform resulted from the greater atrophy of type IIB fibres following denervation. As the changes in MHC isoforms in this study were very small, it is difficult to find a relationship between MHC isoforms and FGFs.

Finally, our results suggest that the ablation of synergists in the plantaris muscle during denervation, the model of passive stretching independent of neural input, gave rise to muscle hypertrophy, and that the hypertrophy may be associated with certain FGFs and HGF. The differential mRNA expression patterns of FGFs and HGF observed following compensatory overload and/or denervation suggest distinct roles for individual FGFs and HGF in muscle hypertrophy and atrophy.

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