Acta Biochimica Polonica

Vol. 43 No. 4/1996

693–700

QUARTERLY

Activity of μ- and m-calpain in regenerating fast and slow twitch skeletal muscles*

Jerzy Moraczewski[a], Ewa Piekarska, Małgorzata Zimowska and Magdalena Sobolewska

Department of Cytology, Institute of Zoology, University of Warsaw, Poland

Received: 6 September, 1996

Key words: calpains, muscle, regeneration

Calpains — non-lysosomal intracellular calcium-activated neutral proteinases, form a family consisting of several distinct members. Two of the isoenzymes: μ (calpain I) and m (calpain II) responded differently to the injury during complete regeneration of *Extensor digitorum longus* (EDL) muscle and partial regeneration of Soleus muscle.

In the crushed EDL the level of m-calpain on the 3rd and 7th day of regeneration was higher than in non-operated muscles, whereas the activity of this calpain in injured Soleus decreased. The level of μ-calpain in EDL oscillated irregularly during regeneration whereas in Soleus of both injured and contralateral muscles its level rapidly rose.

Our results support the hypothesis that m-calpain is involved in the process of fusion of myogenic cells whereas μ-calpain plays a significant but indirect role in muscle regeneration.

Muscle regeneration has been studied for a long time. Mammalian skeletal muscle shows a remarkable ability to regenerate following various kinds of injury. It has been concluded that the process of regeneration is similar essentially, regardless of the cause of injury or disease [1]. The regeneration occurs in two steps: myolysis and reconstruction. During myolysis, intrinsic degradation precedes the breakdown of the damaged myofibres. This process begins with disappearance of Z-lines [2]. α-Actinin, C-protein, troponin, tropomyosin and desmin are cleaved by proteinases called calpains [3].

Muscle regeneration recapitulate embryonic myogenesis: mononucleated myogenic cells proliferate and fuse to form the myotube. Satellite cells, which are monucleated and lie beneath the basal laminae of the adult muscle fibre, are the source of these myogenic cells.

Calpains (EC 3.4.22.17) are non-lysosomal intracellular calcium activated neutral proteinases (CANPs) [4], first characterised in pig muscle [5]. Calpains form a family consisting of several distinct members [6]. They can be divided into two groups: ubiquitous and tissue-specific. Current knowledge on the en-

*This work was supported by the State Committee for Scientific Research (KBN, grant 6 P204 038 05).

[a]To whom correspondence should be addressed: Department of Cytology, Institute of Zoology, University of Warsaw, Krakowskie Przedmieście 26, 00-927 Warszawa 64, Poland, tel. 6200381 ext. 438, fax 268624, e-mail jmlabo@plearn.edu.pl.

Abbreviations: BSA, bovine serum albumin; CANP, calcium-activated neutral proteinase; DE-52, di- ethylaminoethyl cellulose; EDL, *Extensor digitorum longus;* FITC, fluorescein isothiocyanate; PDGF, platelet-derived growth factor; PKC, Ca^{2+}-sensitive, phospholipid-dependent protein kinase; TTBS, Tween/ /Tris buffered saline; $pp39^{mos}$, cytoplasmic serine-treonine protein kinase.

zymic and biological properties of calpains concerns only two of the ubiquitous enzymes present in animal cells. They are designated: μ-calpain (calpain I) and m-calpain (calpain II). These thiol-proteinases are Ca^{2+}-dependent with a half-maximum activity at 1–70 μM Ca^{2+} for μ-calpain and 100–800 μM for m-calpain. Mg^{2+} co-activates these enzymes [7]. Recently a third specie of the ubiquitous type was identified in chicken muscle [8].

Five calpains are heterodimers composed of a large catalytic subunit and identical 30 kDa regulatory subunit present in all the calpain family members. The μ- and m-calpain enzyme systems, the former consisting of the enzyme and its inhibitor, have been identified in several eukaryotic tissues including skeletal muscle, in both vertebrates and invertebrates [9]. Calpains participate in numerous cellular functions through limited hydrolysis of various native proteins [8] cleaving a defined number of specific sites and never releasing small peptides or amino acids. Almost all cytoskeletal proteins, several receptors, such as receptor of platelet-derived growth factor (PDGF), L-type Ca^{2+}-channel proteins as well as some enzymes (e.g. protein kinase C, $pp39^{mos}$, phosphorylase kinase), are known to be calpain substrates [10]. Recent studies have shown that both μ- and m-calpain are widely distributed within an animal cell. Their widespread presence, the character of substrates and the nature of activity suggest that calpains may play a fundamental role in the living cell but it is unclear what is the advantage for a cell to generate two isoenzymes so similar in their activities [11].

Subcellular localization of μ- and m- calpains in muscles was reviewed by Kumamoto *et al.* [12].

Four members of the calpain family (μ, m, μ/m and p94) are expressed in skeletal muscle [6]. The newly discovered calpain isoenzyme p94, is expressed exclusively in skeletal muscles. This isoenzyme is present in muscle usually below detectable levels because it seems to undergo degradation immediately after its being translated [8].

It has been evidenced that these enzymes play an important role in the process of myofibrillar protein turnover [13]. They participate in the postmortem meat tenderization [14]. Also it has been shown that the level of Ca^{2+}-activated proteinases dramatically increased at the early stage of myogenesis [15].

In an attempt to clarify the roles of several calpains in muscle regeneration, we have examined their activities in the fast and slow twitch regenerating rat muscles.

MATERIAL AND METHODS

Regeneration experiments. Adult white Wistar rats (3 months old) were anaesthetized with ether, the EDL or Soleus muscles were denervated and crushed as described in [16]. The non-operated and contralateral muscles were used as controls. After 3, 5, 7 or 14 days of regeneration, the animals were euthanized in ether and both the regenerating and contralateral muscles were removed and weighed.

Calpains were isolated and then activity mesured as described by Savart *et al.* [17] with some modifications.

Preparation of crude extract. Muscles were homogenized in 5 vol. of 20 mM Tris/HCl buffer, pH 7.5, containing 2 mM EDTA, 10 mM EGTA, 0.25 M saccharose, 1% Triton X-100, 10 mM 2-mercaptoethanol and 100 μg/ml leupeptin, using a Kinematica AG PT 1200 Warring blender with two bursts of 30 s and at a 30 s interval. Crude extracts were centrifuged at $10000 \times g$ for 10 min and the obtained supernatants were filtered through glass wool. All operations were performed in an ice bath.

Partial purification of calpains. The supernatant was loaded onto DE-52 columns previously equilibrated with 20 mM Tris/HCl buffer, pH 7.5, containing 2 mM EDTA and 2 mM EGTA. The calpains were eluted with increasing concentration of NaCl in the same buffer. Both μ-calpain and its inhibitor, calpastatin, were eluted with 0.2 M NaCl while m-calpain with 0.4 M NaCl. In the 0.2 M NaCl fraction the concentration of NaCl was adjusted to 1 M and calpastatin and μ-calpain were further separated on a phenyl-Sepharose CL-4B column. The enzyme being finally eluted from column with 20 mM Tris/HCl buffer, pH 7.5, containing 2 mM EDTA, 2 mM EGTA and 1% ethylene glycol. The whole procedure was performed at 4°C.

Assay of calpain activity. 20 mM Tris/HCl buffer containing 6.8 mg/ml FITC-casein and 5 mM $CaCl_2$, or 3 mM EGTA (control sample) was incubated with a μ- or m-calpain fraction for 30 min at 25°C. The reaction was terminated

with 10% trichloroacetic acid at 4°C. After centrifugation at 3600 × *g* for 10 min, the fluorescence of the supernatant was measured in Shimadzu spectrofluorophotometer at 495 nm (excitation) and 520 nm (emission).

Specific activity was expressed as fluorescence units per mg muscle per min. The protein concentration was determined by the method of Bradford [18].

Gel electrophoresis and immunoblotting. Proteins were analysed by SDS/PAGE on 11% polyacrylamide slab gel, according to Laemmli [19]. The calpains separated by electrophoresis were transferred onto a nitrocellulose sheet (Western blot) and detected with anti-calpain polyclonal antibody (generous gift from Dr M. Spencer [20]) and anti-μ-calpain monoclonal antibody (Chemicon, U.S.A.) by indirect immunoperoxidase staining. The Western blots were pre-treated with 5% skim milk or 1% bovine serum albumin (BSA) and 0.1% Tween 20 in Tris buffered saline (TTBS) and were incubated for 30 min at room temperature with primary antiserum diluted 1/200. The blots were then washed with 3 changes of TTBS, followed by 30 min incubation with biotinylated anti-rabbit or anti-mouse IgG antibody (Sigma) diluted 1/10000. After 3 washings (15 min) with TTBS, the blots were incubated in solution of avidin with biotinylated peroxidase ELITE (Vector, U.S.A.). Finally, the blots were treated with peroxidase substrate kit (Vector) and washed with water.

RESULTS

Calpain activity was determined on the 3, 7, and 14th day of regeneration of crushed, contralateral, and non-operated muscles. Regeneration progressed differently in EDL and in Soleus. EDL regenerated quickly and completely whereas Soleus regenerated only partially and sometimes not at all. In EDL normal structure was totally recovered after 16 days after the injury while regeneration in Soleus, although started at first, soon stopped and muscle became fibrotic [16].

The presence of calpains in the extracts from non-operated EDL and Soleus muscles was evidenced using two different types of antibodies (Fig. 1). The polyclonal antibody, as it was previously shown by Spencer [20] recognized all three isoforms of muscle calpains (m, μ, p94). Consistently, Western blotting revealed three bands: one of 100 kDa, corresponding probably to the specific muscle p94 calpain, and two other overlapping bands of about 80 kDa (Fig. 1A). The monoclonal antibody against μ-calpain large subunit (Chemicon), which does not react with m-calpain, gave only one band with either muscle extract (Fig. 1B).

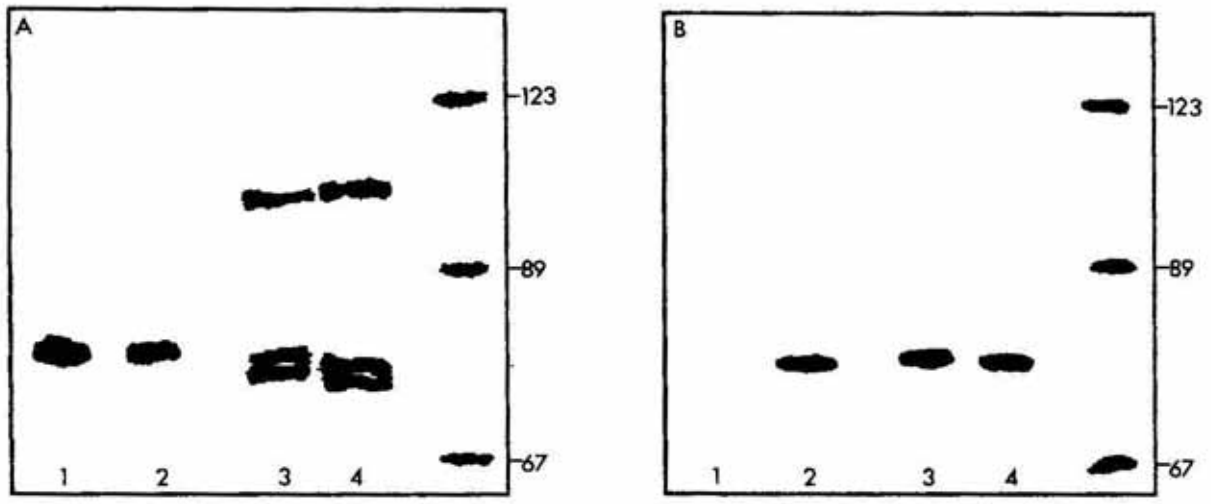

Fig. 1. Immunoblots of calpains (Calbiochem) and calpains from non-operated EDL and Soleus muscles (A) with polyclonal antibody, and (B) with monoclonal anti-μ-calapin antibody.
Comercial m-calpain (lane 1), and μ-calpain (lane 2); calpains from non-operated EDL (lane 3), and from non-operated Soleus (lane 4). Molecular mass markers: β-galactosidase (123 kDa), fructose-6-phosphate kinase (89 kDa), pyruvate kinase (67 kDa) (Sigma products).

In the EDL extract from non-operated muscle (day 0) the activity of m-calpain was 81 ± 9 units. In the injured muscle it was doubled as early as on the 3rd day (189 ± 9 units) and more than tripled on the 7th day (294 ± 8 units) of regeneration. After 14 days the m-calpain activity dropped below the level detected in non-operated muscle. In the contralateral muscle slight, insignificant fluctuations of m-calpain level took place between the 3rd and 7th day of the experiment, followed by a marked decrease after 14 days, thus resembling the decrease of the activity in regenerating muscle. It should be noted that in contralateral EDL muscle the activity was from 2 to 3 times lower than in the crushed muscle. In the non-operated Soleus muscle a high level of the m-calpain isoform was detected (189 ± 26 units), twice as high as in EDL (Fig. 2B), but it was reduced to about 40% of the control value on the 3rd day after injury. In the following days the activity was gradually increasing and on the 14th day of the experiment it amounted to 229 ± 23 units, but it remained significantly lower than in EDL. In contrast to EDL, the activity in contralateral Soleus muscle was as high as in the crushed muscle or even somewhat higher on the 3rd day after crushing. After 14 days the activity in the crushed Soleus became twice as high as in the contralateral muscle.

The level of μ-calpain in both non-operated muscles was similar and amounted to about 28 units. In regenerating EDL the level of this proteinase was fluctuating; an increase observed after 3 days was followed by a decrease on the 7th day and an increase 14 days after the injury (Fig. 3A and B). On the 7th day of EDL regeneration, i.e. at the time of the highest m-calpain activity, the level of the μ-isomer was the lowest. At variance with EDL crushed muscle, μ-calpain activity in Soleus was raised 4- and 7-fold on the 3rd and 7th day of the experiment respectively, and reached a plateau after 14 days. The time course of changes in μ-calpain level in the contralateral muscle followed that in the crushed muscle (Fig. 3B).

A comparison of calpains activities in the injured muscles to the activities before crushing shows not only distinct differences in the response of the two muscles to the injury, but also implies a different effect of injury on the contralateral muscle (Fig. 4). One can see that the effect of crushing was stronger in the contralateral Soleus muscle, than in the EDL muscle.

DISCUSSION

Mature skeletal muscles constitute a mosaic of slow- and fast-twitch fibers. Fast relaxation is characteristic of white fibers, whose cells contain small amounts of myoglobin and few mitochondria. These fibers generate energy by

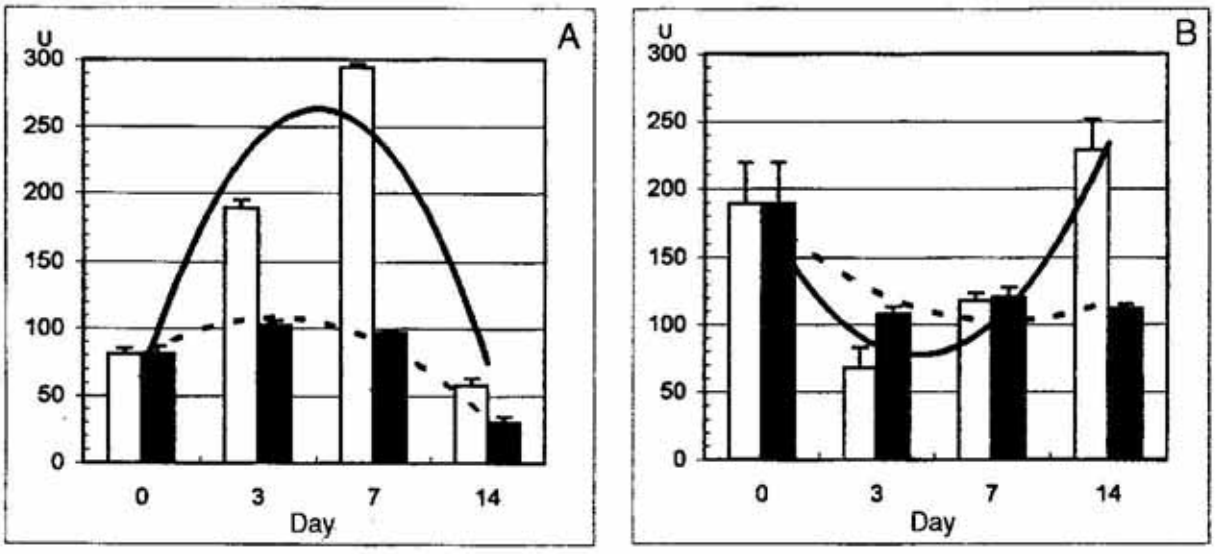

Fig. 2. The level of m-calpain in regenerating (open columns) and contralateral (closed columns) EDL (A) and Soleus (B) muscles.

Polynomial tendence curve of the values in regenerating (solid line) and contralateral (broken line). Each value is the mean from the extracts obtained from 6 animals, assayed in triplicate.

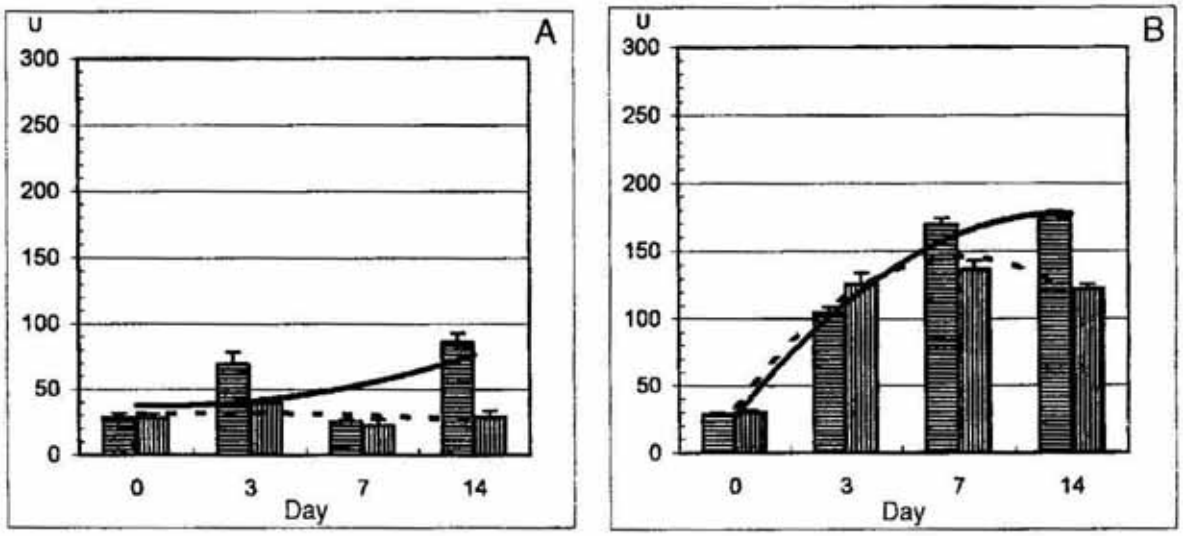

Fig. 3. The level of μ-calpain in regenerating (cross striated columns) and contralateral (longitudinal striated columns) EDL (A) and Soleus (B) muscles.

means of anaerobic glycolysis. Red muscle fibers, which relax slower and are more resistant to fatigue, are rich in myoglobin and mitochondria. For these fibers, oxidative phosphorylation is the energy-generating process. Rat EDL contains about 95% of "fast" fibers, whereas Soleus is in 80–100% composed of "slow" fibers [16]. Therefore, EDL could well be referred to as the fast-twitch muscle, and Soleus as the slow-twitch muscle. Another difference between these two types of muscle is their different ability to regenerate after being crushed. EDL regenerates quickly and completely, and after 14 days following the injury it already contains new fibers and innervation. Soleus restores its fibers hardly or even not at all and by the 14th day following the injury it undergoes degeneration manifested by overgrowth of connective tissue [16].

As we have demonstrated previously [21], protein kinase C (PKC) is involved in differentiation of muscle cells during regeneration and

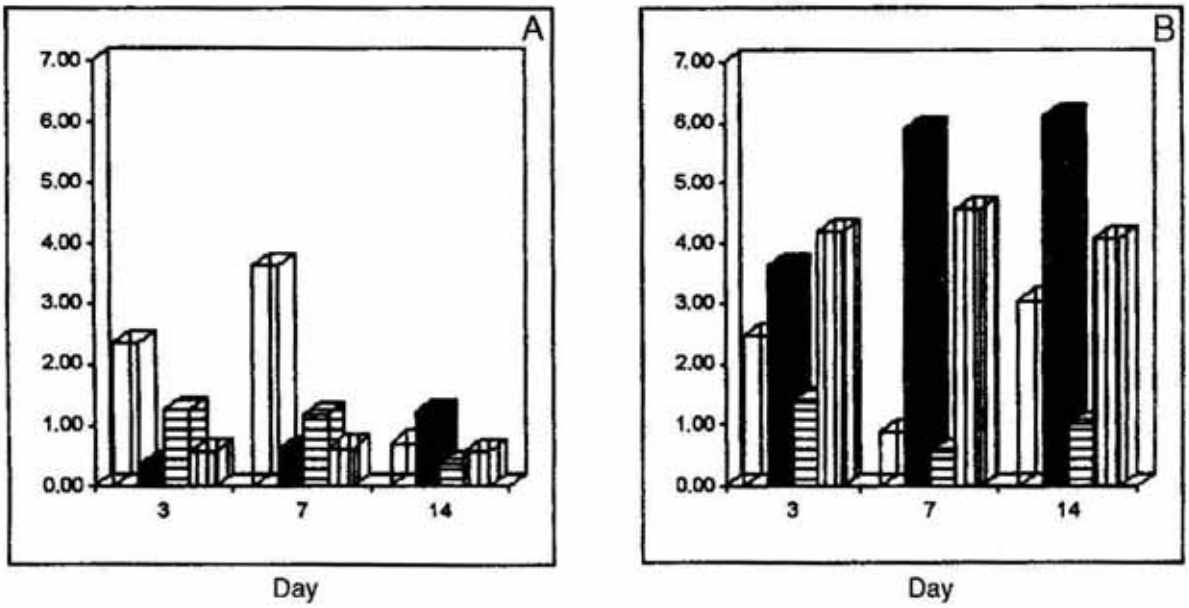

Fig. 4. The relative level of m-calpain (A) and μ-calpain (B) in regenerating (open columns) and contralateral (closed columns) EDL and in regenerating (cross striated columns) and contralateral (longitudinal striated columns) Soleus muscles vs non-operated muscles.

embryonic development. Taking into account that calpain is one of the major activators of PKC and, according to Savart *et al.* [22], a stable and functional PKC-μ-calpain complex exists in skeletal muscles, we have decided to investigate changes in calpain levels in regenerating and contralateral muscles.

Despite the major role played by the two investigated calpain isoforms in muscles [10], their levels in this tissue are considerably lower than in other tissues or organs [23]. The activities of both m- and μ-calpain in rat spleen were twice and five times as high as those in muscle, respectively (not shown). An extremely high activity of m-calpain in Soleus muscle from non-operated rats should be emphasized. Other authors also found, in pigs, that m-calpain was more abundant in the "slow" muscle than in the "fast" muscle [24]. The response of m-calpain activity to the injury in the two muscles was distinctly different. During EDL regeneration the level of m-calapin increased concurrently with the key changes occuring in the regenerating muscle. The 3rd day of the regeneration marked the beginning of the differentiation of new muscle cells and day 7 marked the beginning of most dynamic cell fusion and myotube formation (not shown). As can be seen (Fig. 2A) m-calpain was most abundant during formation of muscle fibers. Brustis *et al.* [25] provide evidence that extracellular m-calpain is essential for myoblast fusion. It has been previously demonstrated [26] that in the *in vitro* regenerating myoblasts the level of m-calpain is the highest at the initial stage of cell fusion. Our present results support the assumption that m-calpain is involved in the process of fusion of myogenic cells.

In contrast to EDL in Soleus muscle, a reverse time course of m-calpain level was observed. Polynomial tendency curve (Fig. 2B) showed the minimum at the time when myoblast fusion took place. On the 3rd day of regeneration Soleus muscle was still swollen and filled with blood, and on the 7th day myoblast fusion could be observed only sporadically [16]. Soleus muscle from non-operated rats is very rich in m-calpain, consistently with the results of Elce *et al.* [27] and Kawashima *et al.* [23]. There may be two reasons for the decrease in the level of this enzyme during the first three days after crushing of muscle (Fig. 2B): proteolysis or autolysis of the enzyme and/or lack of its synthesis in damaged tissue.

Elce *et al.* [27] found a relation between the activities of Ca^{2+}-dependent proteinases in denervated and contralateral rat Soleus muscles. Their results, and our data on PKC in regenerating muscles (unpublished), indicate that a muscle of one limb reacts to an operation performed on the corresponding muscle in the matching limb. Apparently, certain enzymes respond to the operational stress by changing their activity or the level of their synthesis (Gautron, personal information). Parr *et al.* [28] demonstrated that stimulation of β-adrenergic receptors in muscles resulted in an increase in the synthesis of m-calpain and its inhibitor, calpastatin, as well as in reduction of μ-calpain synthesis. Our data are consistent with these conclusions. However, the effect of injury on the contralateral muscle was different. In EDL the activity of both m- and μ-calpains in contralateral muscle was of the same order of magnitude as in the non-crushed muscle, while the activity in Soleus muscle generally followed the same trend as in crushed muscle (Fig. 4). Our results lead us to believe that the analysis of changes in calpain level should be based not on a comparison of the changes occurring in regenerating *vs* contralateral muscles, since both types of muscle undergo different processes in response to the operation. The only valid comparison seems to be between muscles from operated and non-operated rats.

In our opinion, μ-calpain does not play a significant, direct role at the early stage of muscle regeneration as does m-calpain. Some conclusions can be drawn from observing the changes in the level of μ-isoform in regenerating muscles *vs* muscles from non-operated rats, and from comparing the fast twitch muscle with the slow twitch one. The level of μ-calpain in the muscles of non-operated animals is the same in both EDL and Soleus but, contralateral EDL hardly reacts at all to the operation, while operated EDL shows an increase in μ-calpain activity on the days when m-calpain activity is low and *vice versa*. Both in contralateral and operated Soleus a very strong activation of μ-calpain was noted. Possibly μ-calpain is less prone to undergo degradation since its level did not decrease below that detected in the muscles from non-operated rats. An increase in μ-cal-

pain activity could be due to the presence of blood cells such as platelets or granulocytes. In myoblasts cultured *in vitro* [24] μ-calpain is synthesized throughout the whole period of cell growth and differentiation and its level rises systematically. It is hard to define at this moment the role played by this isoenzyme in the complex process of regeneration. Moreover, the use of polyclonal antibodies against calpain did not yield clear results. Three bands were obtained (Fig. 1), one of which was situated near a standard protein of 100 kDa and thus could correspond to the new p94-calpain typical of the muscle tissue [13, 29]. Alternatively, this band should be ascribed to yet another calpain, namely the μ/m one. The development of specific antibodies and determination of individual mRNAs seems to be necessary for elucidation of the role played by particular calpain isoform during muscle regeneration.

REFERENCES

1. Campion, D.R. (1984) The muscle satellite cell: A review. *Intern. Rev. Cytol.* **87**, 225–251.
2. Grounds, M.D. (1991) Towards understanding skeletal muscle regeneration. *Path. Res. Prac.* **187**, 1–22.
3. Goll, D.E., Kleese, W.C., Okitani, A., Kumamoto, T., Cong, J. & Kaprell, H.-P. (1990) Historical background and current status of the Ca^{2+}-dependent proteinase system; in *Intracellular Calcium-Dependent Proteolysis* (Mallgren, R.L. & Murachi, T., eds.) pp. 3–24, CRC Press, Boca Raton, FL.
4. Suzuki, K., Saido, T.C. & Shuichi, H. (1992) Modulation of cellular signals by calpain. *Ann. NY Acad. Sci.* **674**, 218–227.
5. Dayton, W.R., Reville, W.J., Goll, D.E. & Stromer, M.H. (1976) A Ca^{2+} activated protease possibly involved in myofibryllar turnover: Partial characterization of the purified enzyme. *Biochemistry* **15**, 2159–2167.
6. Siromachi, H., Saido, T.C. & Suzuki, K. (1994) New era of calpain research. Discovery of tissue-specific calpains. *FEBS Lett.* **343**, 1–5.
7. Suzuki, K. & Ohno, S. (1990) Calcium activated neutral protease structure function relationship and functional implicatons. *Cell Struc. Funct.* **15**, 1–6.
8. Sorimachi, H., Toyama-Sorimachi, N., Saido, T.C., Kawsak, H., Sugita, H., Miyasaka, M., Arahata, K., Ishiura, S. & Suzuki, K. (1993) Muscle specific calpain, p94, is degraded by autolysis immediately after translation, resulting in disappearance from muscle. *J. Biol. Chem.* **268**, 10593–10605.
9. Meloni, E. & Pontremoli, S. (1989) The calpains. *Trends Neurosci* **12**, 438–444.
10. Johnson, P. (1990) Calpains (intracellular calcium-activated cysteine proteinases): Structure--activity relationships and involvement in normal and abnormal cellular metabolism. *Int. J. Biochem.* **22**, 811–822.
11. Kwak, K.B., Chung, S.S., Kim, O.M., Kang, M.S., Ha, D.B. & Chung, C.H. (1993) Increase in level of m-calpain correlates with the elevated cleavage of filamin during myogenic differentiation of embryonic muscle cells. *Biochim. Biophys. Acta* **1175**, 243–249.
12. Kumamoto, T., Kleese, W., Cong, J., Goll, D.E., Pierce, P.R. & Allen, R.E. (1992) Localization of Ca^{2+}-dependent proteinases and their inhibitor in normal, fasted, and denervated rat skeletal muscle. *Anat. Rec.* **232**, 60–77.
13. Goll, D.E., Thompson, V.F., Taylor, R.G. & Christiansen, J.A. (1992) Role of the calpain system in muscle growth. *Biochimie* **74**, 225–237.
14. Goll, D.E., Otsuka, Y., Nagainis, A., Shannon, J.D., Sathe, S. & Maguruma, M. (1983) Role of muscle proteinases in maintenance of muscle integrity and mass. *J. Food Biochem.* **7**, 137–177.
15. Scholmayer, J.E. (1986) Role of Ca^{2+} and Ca^{2+}--activated proteases in myoblast fusion. *Exp. Cell Res.* **162**, 411–422.
16. Bassaglia, Y. & Gautron, J. (1995) Fast and slow rat muscles degenerate and regenerate differently after whole crush injury. *J. Muscle Res. Cell Motility* **16**, 420–429.
17. Savart, M., Belamri, M., Pallet, V. & Ducastaing, A. (1987) Association of calpains 1 and 2 with protein kinase C activities. *FEBS Lett.* **216**, 22–26.
18. Bradford, M.M. (1976) A rapid and sensitive method for the quantification of microgram quantity of protein utilizing the principle of dye binding. *Anal. Chem.* **72**, 248–254.
19. Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* **227**, 680–685.
20. Spencer, M.J., Croall, D.E. & Tidbald, J.G. (1995) Calpains are activated in necrotic fibers from mdx dystrophic mice. *J. Biol. Chem.* **270**, 10909--10914.
21. Moraczewski, J., Martelly, I., Trawicki, W., Pilarska, M., Le Moigne, A. & Gautron, J. (1990) Activity of protein kinase C during development in rat skeletal muscle. *Neurosci. Res. Commun.* **7**, 183–189.

22. Savart, M., Verret, C., Dutaud, D., Touyarot, K., Elamrani, N. & Ducastaing, A. (1995) Isolation and identification of a μ-calpain-protein kinase C α complex in skeletal muscle. *FEBS Lett.* **359**, 60–64.

23. Kawashima, S., Hayashi, M., Saito, Y., Kasai, Y. & Imahori, K. (1988) Tissue distribution of calcium-activated neutral proteinases in rat. *Biochim. Biophys. Acta* **965**, 130–135.

24. Kim, S.Y., Sainz, R.D. & Lee, Y.-B. (1993) Note on the comparison of calpains I, II and calpastatin activity in two different types of porcine skeletal muscles. *Comp. Biochem. Physiol.* **105A**, 235–237.

25. Brustis, J.-J., Elamrani, N., Balcerzak, D., Sawate, A., Soriano, M., Poussard, S., Cottin, P. & Ducastaing, A. (1994) Rat myoblast fusion requires exteriorized m-calpain activity. *Eur. J. Cell. Biol.* **64**, 320–327.

26. Moraczewski, J., Piekarska, E., Bonavaud, S., Wosinska, K., Chazud, B. & Barlovatz-Meimon, G. (1996) Different intracellular distribution and activities of μ- and m-calpains during the differentiation of human myogenic cells in culture. *C.R Acad. Sci. Paris* **319**, 681–686.

27. Elce, J.S., Hasspieler, R. & Boegman, R.J. (1983) Ca^{2+} activated protease in denervated rat skeletal muscle measured by an immunoassay. *Exp. Neurol.* **81**, 320–329.

28. Parr, T., Bradsley, R.G., Gilmour, R.S. & Buttery, P.J. (1992) Changes in calpain and calpastatin mRNA induced by β-adrenergic stimulation of bovine skeletal muscle. *Eur. J. Biochem.* **208**, 333–339.

29. Siromachi, H. & Suzuki, K. (1992) Sequence comparison among muscles-specific calpain, p94 and calpain subunits. *Biochim. Biophys. Acta* **1160**, 55–62.