Calcineurin Is Required for Skeletal Muscle Hypertrophy*

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Shannon E. Dunn, Jennifer L. Burns, and Robin N. Michel‡

From the Department of Chemistry and Biochemistry, Neurornuscular Research Laboratory, Laurentian University, Sudbury, Ontario P3E 2C6, Canada

Molecular signaling pathways linking increases in skeletal muscle usage to alterations in muscle size have not been identified. In the present study, we tested the hypothesis that calcineurin, a calcium-regulated phosphatase recently implicated in the signaling of some forms of cardiomyopathic growth, is required to induce skeletal muscle hypertrophy and muscle fiber type conversions associated with functional overload in vivo. Administration of the specific calcineurin inhibitors cyclosporin (CsA) or FK506 to mice, for which the fast plantaris muscle was overloaded for 1–4 weeks, prevented the rapid doubling of mass and individual fiber size and the 4–20-fold increase in the number of slow fibers that characterize this condition. CsA treatment influenced the expression of muscle myofibrillar protein genes in a way reflective of fiber phenotype transformations but only in the long term of the overload condition, suggesting that the control of this growth response by calcineurin is not limited to the transcriptional activation of these muscle-specific genes. Clinically, these results provide insight to the post-surgical muscle wasting and weakness observed in recovering transplant recipients administered therapeutic dosages of these immunosuppressants.

The amount and type of contractile proteins incorporated into the myofibril of skeletal muscle fibers are major determinants of the size, strength, and speed of these cells (1). To date, the molecular events linking muscle usage to the cellular expression and accumulation of these proteins are unknown. Recently, calcineurin, a cytoplasmic calcium-regulated phosphatase implicated in the pathogenesis of hypertrophic cardiomyopathy (2, 3), has emerged as a possible candidate in the signaling of skeletal muscle cellular growth and the fiber type transformations (4) of these cells. Calcineurin is an enticing prospect as a regulatory enzyme in this signaling because its selective activation of NF-AT (nuclear factor of activated T cells) transcription factors in response to sustained increases in intracellular calcium concentrations (5) is reminiscent of calcium fluctuations provoked by the activation of muscle cells during extensive contractile work (4, 6).

Typically, a fast weight-bearing muscle subjected to the functional loss of its synergists will compensate by displaying within 2–4 weeks a doubling of mass and individual fiber sizes and an increase in strength (7, 8). A muscle overloaded in this manner will also contract more slowly as a result of rapid fiber type transformations characterized by an increase in the number of fibers exhibiting slower, more energy-efficient contractile and calcium-handling proteins (7–9). In rodent fast muscle, the myosin heavy chain (MHC)1 enzyme component of the major contractile protein myosin displays a conversion pattern in response to overload that follows from the fastest to the slowest isoform in the order MHC Iib → IIX → IIsa → Islow. The signaling of these adaptations may well be mediated by calcineurin since most of the functional indices of overloaded muscles (8) suggest that fiber intracellular calcium levels are chronically elevated in these tissues. Thus we tested the hypothesis that administration of CsA or FK506, both specific inhibitors of calcineurin (10), to adult mice, for which the fast plantaris hind limb muscle was subjected to compensatory functional overload, would prevent the ensuing hypertrophy and MHC-type conversions that characterize this model. Preliminary results have appeared in abstract form (11).

EXPERIMENTAL PROCEDURES

Plantaris Overload and Drug Administration—The plantaris in each hind limb was overloaded (OV) via surgical removal of soleus and gastrocnemius muscles (7) in male CD-1 mice (22–28 g) injected with either CsA (25 mg/kg, subcutaneously), FK506 (3–5 mg/kg, subcutaneously), or vehicle (cremophor EL) twice daily (2) for 1, 2, or 4 weeks. This dose of CsA is higher than that reported to inhibit 90% of total calcineurin phosphatase activity in the heart, to block Ca2+-induced NF-AT dephosphorylation in spleen cell lysates, and to inhibit calcineurin-mediated transcriptional activation in skeletal muscle (3, 12, 13). Our treatment resulted in blood levels of CsA that were 1682 ± 308 (n = 7) ng/ml 6 h after the last injection. Blood levels for FK506 were 83 ± 14 (n = 4) and 262 (n = 1) ng/ml for each respective dose (Isotechnika, Edmonton, Alberta). A separate group of OV animals (n = 4) were also administered rapamycin (Alexis, San Diego, CA; 2 mg/kg in 2.5% Tween 80, 5% N,N-dimethylacetamide, 17.5% polyethylene glycol in saline, subcutaneously once daily) over a 2-week period which resulted in blood levels for this drug of 208 ± 10 ng/ml (Isotechnika). For OV animals, access to food was adjusted to promote usage of the hind limb musculature during feeding. Sham-operated mice administered vehicle or CsA served as controls. Injection of these chemical agents did not affect the health (from general observations and autopsy results), growth (body weights were not different among mice at any time during treatment), or noticeably alter the daily amount of locomotor activity displayed by experimental animals. The efficacy of ankle plantar flexion was verified twice daily using established functional criteria (14) and was found to be noticeably weaker in overloaded mice administered calcineurin inhibitors at treatment end points compared with overload-vehicle counterparts. At the appropriate end points, muscles were excised, weighed, and quick frozen in melting isopentane precooled with liquid nitrogen. All surgical procedures were performed under sterile conditions on mice anesthetized (1.2 μg/g intramuscularly) with 100 mg/ml ketamine hydrochloride, 20 mg/ml xylazine in a volume ratio of 1:6:1. Treatment of animals was in accordance with guidelines established by the Canadian Council on Animal Care.

Immunolabeling of MHC and Fiber Size Analysis—Cryosections

1 The abbreviations used are: MHC, myosin heavy chain; CsA, cyclosporin A; OV, overloaded; BrdUrd, 5-bromo-2′-deoxyuridine; RT-PCR, reverse transcription-polymerase chain reaction; bp, base pair(s); TNs, slow troponin I; TnI1, fast troponin I.

2 This paper is available on line at http://www.jbc.org

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‡ To whom correspondence should be addressed: Dept. of Chemistry & Biochemistry, Laurentian University, Ramsey Lake Rd., Sudbury, Ontario P3E 2C6, Canada. Tel.: 705-675-1151 (Ext. 1010); Fax: 705-675-4844; E-mail: rmichel@nickel.laurentian.ca.

3 The abbreviations used are: MHC, myosin heavy chain; CsA, cyclosporin A; OV, overloaded; BrdUrd, 5-bromo-2′-deoxyuridine; RT-PCR, reverse transcription-polymerase chain reaction; bp, base pair(s); TnIs, slow troponin I; TnI1, fast troponin I.
were cut from the same anatomical location in the midbelly of each plantaris muscle. Tissue sections were probed with mouse antibodies raised against embryonic (F1.652), I (BA-F8), Ila (SC-71), Iib (BF-F3), and all MHCs except Ix (BF-35), followed by peroxidase-conjugated goat anti-mouse IgG, or IgM in the case of BF-F3 (7). Bound antibodies were visualized using diaminobenzidine tetrahydrochloride. Fibers expressing or coexpressing I, Ila, Iib, or exclusively expressing Ix MHC were identified within three distinct tissue fascicles of each muscle, and their cross-sectional size was measured using a microscope linked to a computer-based image analysis system as described previously (7).

Labeling of Cell Nuclei—As a post hoc consideration, the proliferation of satellite cell and non-muscle nuclei in plantaris muscles of 5-day treated OV-veh and OV-CsA mice was assessed by labeling their uptake of the thymidine analog 5-bromo-2′-deoxyuridine (BrdUrd) as described previously (15). Briefly, a mini-osmotic pump (model 1007D, Alza Corp., Palo Alto, CA) containing BrdUrd (25 mg/ml) was implanted along the dorsal midline of 3 Sham-veh, OV-veh, and OV-CsA mice and delivered BrdUrd systemically at a continuous rate of 0.5 μl/h for the 5-day duration of the experiment. Muscle nuclei (pre-existing myonuclei plus satellite cell nuclei) were stained in cryosections using the BrdUrd antibody G34G and delineated from non-muscle nuclei by staining the extracellular matrix with the laminin antibody 2E8 (15). Both antibodies were obtained from the Developmental Studies Hybridoma Bank, University of Iowa.

Quantitative Reverse Transcription-Polymerase Chain Reaction (RT-PCR)—Total RNA was extracted from analogous distal portions (i.e. devoid of any remnant gastrocnemius fibers) of each plantaris muscle, and 2 μg of each sample was reverse-transcribed as described (16). The RT-negative control consisted of total RNA plus RT mixture minus the addition of reverse transcriptase. Amplification of MHC, slow troponin I (TnIs), fast troponin I (TnIf), and 28 S ribosomal RNA cDNAs was achieved using specific primers designed as follows. For amplification of MHC I, IIa, IIb, and Ix cDNAs, we used a common 5′ primer designed from the rat cDNA sequence (5′ ACJ04993) and were 5′ to mRNA positions 93–103) and 5′ complementarity regions of these genes: I (5′ CTTACTCTTCATTAGTTCA5′, complementary to cDNA positions 533–552), IIa (5′ CCTTACTCTTCATTAGTTCA5′, complementary to cDNA positions 528–551), Ibx (5′ ATCTCTGTGGCTAATTCGTCA5′, complementary to cDNA positions 560–582), and Iib (5′ TAGCTTTTCATCTAATTCGTCA5′, complementary to cDNA positions 351–373) (17). TnI 5′ and 3′ primers were designed from the rat mRNA sequence (GenBank TM/EBI ACJ04993) and were 5′ GATCTCTTCATTAGTTCA5′ (corresponding to mRNA positions 93–103) and 5′ GAACATCTTCTGCGACCTTC5′ (corresponding to mRNA positions 557–577), respectively. TnIf 5′ and 3′ primers were designed from the mouse mRNA sequence (GenBank TM/EBI ACJ04992) and were 5′ GAAGAGAAGCTACTCTGCAAG5′ (corresponding to mRNA positions 143–163) and 5′ TGGGGCAGTTAGACCTGACGTC5′ (corresponding to mRNA positions 558–579). Ribosomal 28 S RNA subunit 5′ and 3′ primers designed from the rat cDNA sequence (GenBank TM/EBI ACJ11167) were 5′ TGTGTTGCCATTGTA-ATCTGGTGTGATACGTC5′ (corresponding to cDNA positions 4535–4564) and 5′ TCTGACATAGGAGGCTTGGCATGCA5′ (corresponding to cDNA positions 5597–5613).

Quantitative PCR analysis was performed as described previously (14). Sense primers were labeled with γ-32P using T4 polynucleotide kinase (Amersham Pharmacia Biotech, Oakville, Ontario). For PCR, 2.5 μl of RT sample was added to a PCR reaction mixture that contained 10 × PCR buffer, 0.65 unit of Taq polymerase (both from Qiagen, Melbourne, Ontario), 10 pmol of each primer, and 5 μl each dNTP (Life Technologies, Inc., Burlington, Ontario). Amplification conditions consisted of a 1-min denaturation at 94 °C, 1-min annealing at 55 °C, and 1-min extension at 72 °C for 11–31 cycles. For each primer set, cycle number was adjusted to permit comparison of PCR products across treatments within their linear phase of amplification. Tubes that contained PCR reagents plus the RT-negative sample or ultrapure water served as controls. PCR products were electrophoresed on 1% agarose gels and visualized with ethidium bromide. For quantification, individual product bands and representative background were excised from each gel lane and subjected to Cerenkov counting (cpm). A DNA 100-bp ladder was used to estimate the length of each PCR product. The identity of each PCR product was also verified using a series of restriction enzymes and DNA sequencing (Biomolecular Sequencing, Charlottesville, VA).

RESULTS AND DISCUSSION

As expected, overloaded mice administered vehicle displayed an increase (90–110%) in mean plantaris mass after 2–4 weeks of this condition compared with Sham-operated counterparts.
(Fig. 1a). This increase in muscle mass in response to overload corresponded to a progressive increase (55–130%) in the mean cross-sectional size of all plantaris MHC-typed fibers over 4 weeks of this treatment (Fig. 1, b and c). Although mean plantaris mass of overloaded mice administered CsA was higher (90%) at 2 weeks, the mean muscle mass of overloaded counterparts administered FK506 was not different from Sham-veh at 2- and 4-week time points (Fig. 1a). Similarly consistent with this drug effect, CsA treatment significantly counteracted the overload-associated increase in mean muscle mass over 4 weeks of this condition (Fig. 1a). More importantly, mean cross-sectional size across all MHC-typed fibers in both OV-CsA- or OV-FK506-treated tissues was not different from controls at either 2 or 4 weeks post-surgery (Fig. 1, b and c), clearly demonstrating the inability of these cells to hypertrophy in the presence of these calcineurin inhibitors despite the increased functional demands imposed by the overload condition. The histological appearance of plantaris fibers was normal at all post-treatment time points. Taken in this light, the increase in mean plantaris whole muscle mass observed in OV-CsA mice at 2 weeks is consistent with the notion that these muscles would be most susceptible to work-related extracellular phenomena (i.e. interstitial edema) sustained as a result of their inability to adapt to the overload condition at the single-fiber level. The fact that this extracellular response was tempered in OV-FK506 animals may relate to the relatively higher potency of this drug as an anti-inflammatory agent over its CsA counterpart (18).

The absence of muscle hypertrophy in overloaded mice administered calcineurin inhibitors was not related to the effects of injection of these drugs alone (Fig. 1, a, b, and c; but see Fig. 1 legend) or to OV-CsA and OV-FK506 animals demonstrating less daily locomotor activity compared with vehicle-injected counterparts (see “Experimental Procedures”). Moreover, the effects of overload and CsA administration were not species-specific since muscle fiber hypertrophy was also prevented in adult rats under similar conditions.2

To determine whether the prevention of hypertrophy by CsA and FK506 was related to immunophilin inactivation, we administered rapamycin to a separate group of overloaded mice (n = 3). Rapamycin is an immunosuppressant drug that complexes with an FK506-binding protein (FKBP12) but does not target calcineurin (19). Similar to the effects of OV-veh and in contrast to those of FK506, administration of rapamycin to OV mice resulted in a significant increase (37–49%) in size of plantaris fibers expressing MHC IIa (860 ± 28 versus 578 ± 45) and IIx (1267 ± 65 versus 924 ± 54) above Sham-veh levels after 2 weeks of this condition, emphasizing that the prevention of hypertrophy in these cell types at this time was likely mediated via calcineurin inhibition. On the other hand, rapamycin did prevent hypertrophy of fibers expressing I MHC (564 ± 32 versus 559 ± 50), a finding that is hard to reconcile but may relate to the fact that rapamycin-FKBP complexes also interfere with mRNA translation via mTOR (mammalian target of rapamycin), an important signaling intermediate for cellular protein turnover (20). The finding that rapamycin may have other yet undetermined effects in vivo is further supported by the observation that OV-rapamycin tissues were extensively degenerative, displaying a distinct and considerable reappearance of small (<350 μm²) de novo fibers that decorated with embryonic MHC compared with FK506 or CsA counterparts (data not shown).

The rapid muscle fiber growth associated with functional overload has been attributed in part to the proliferation of satellite cells and subsequent fusion of their progeny with pre-existing muscle fibers (15). The recent findings that CsA administration prevents the differentiation and fusion of myoblasts in vitro and hinders muscle regeneration in response to injury in vivo (21) raised the possibility that the prevention of hypertrophy in adult OV-CsA-treated muscles is related to an inhibition of satellite cell differentiation under these conditions. To address this, we tested for the presence of fibers expressing embryonic MHC and displaying centrally located nuclei, both hallmarks of muscle regeneration (21), within excised tissue sections after 1–2 weeks of OV-veh and OV-CsA treatments. We detected a comparable number of regenerating fibers after both conditions mainly within remnant portions of the ablated gastrocnemius (data not shown). We also assessed the extent of satellite cell proliferation within OV-veh and OV-CsA plantaris tissues using the thymidine analog BrdUrd and found it to be minimal, suggesting that proliferation of satellite cells is not a major contributor to the rapid growth of pre-existing plantaris fibers in response to overload (data not shown). Taken together, these findings suggest that the prevention of hypertrophy in OV-CsA-treated tissues cannot be ascribed in any major way to mechanisms involving inhibition of satellite cell differentiation or muscle fiber regeneration by CsA.

Also consistent with our hypothesis, fiber conversions that occur in response to functional overload (7) were also prevented by the administration of these specific calcineurin inhibitors. Immunolabeling of plantaris cross-sections with a slow/type I MHC-specific antibody showed that overload induced a quadrupling of the number of fibers expressing this protein after 2 weeks, which progressed to a 20-fold increase in this number by 4 weeks (Fig. 2). These gains in the proportion of fibers expressing I MHC over the 4-week period were at the major expense of pre-existing mature fibers expressing fast MHC isoforms and not the result of a significant generation of new fibers (data not shown; see Ref. 7). These extensive MHC fast/type II → slow/type I fiber transformations did not occur in overloaded mice administered CsA (Fig. 2, c and e). Additionally, immunolabeling of the fast IIa, IIx, and IIb MHCs in serial cross-sections showed that OV mice administered vehicle displayed the IIb → IIx → IIa MHC conversions typical of this condition, whereas these transformations were prevented in CsA-injected counterparts (data not shown). In this respect, our findings in vivo not only support the recent contention that calcineurin may influence the expression of slow contractile proteins in vitro (4) but further these observations by showing that this enzyme also plays a critical role in the expression of fast MHC counterparts during transitions within the fast fiber population itself (i.e. MHC IIb → IIx or IIx → IIa) in response to chronic increases in muscle usage.

Recent studies provide evidence that calcineurin-NF-AT signaling may mediate hypertrophy in cardiac muscle and fast-to-slow phenotypic adaptations in skeletal muscles in vitro, in part, via the transcriptional activation of a subset of slow muscle-specific genes (2–4). This is particularly true of the slow isoform of troponin I which harbors distinct consensus elements for NF-AT in its promoter (22). Indeed, calcineurin-mediated transcriptional activation of slow fiber genes such as Tnls and associated reciprocal repression of its fast fiber counterpart TnIf by NF-AT factors has been proposed as a model to explain fast-to-slow fiber conversions in skeletal muscle in response to chronic increases in muscle activation (4). In light of this, we examined whether the prevention of overload-induced hypertrophy and fiber transitions by calcineurin inhibition were related to changes in the expression of Tnls and MHC genes. To this end we used a semiquantitative RT-PCR assay to

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assess TnI and MHC transcript levels relative to total RNA in treated tissues.

For the most part, overload-induced changes in TnI and MHC transcript levels were more reflective of fast-to-slow fiber conversions than the hypertrophic growth observed across all plantaris cell types. Specifically, we observed increases in TnIs mRNA and reciprocal decreases in TnIf mRNA after 1 (data not shown), 2 (Fig. 3), and 4 (Fig. 4) weeks of overload that were in line with predictions based on the fiber-type transformations associated with this model. A similar reciprocal change in mRNA levels was observed for MHC I and IIb, but only after 4 weeks of overload, suggesting a discordant modulation of the expression of specific myofibrillar genes during the course of this condition. In this sense, our data are consistent with those of others using this same model (23) and suggest that the cellular expression and accumulation of key myofibrillar proteins in the early stages of overload may be mediated, in large part, by post-transcriptional events.

Calcineurin did appear to influence transcription of TnI and MHC genes in a way reflective of fiber conversions but only in the long term of overload, such that increases in TnIs and MHC I and decreases in TnIf and MHC IIb mRNAs were prevented by CsA administration at 4 weeks (Fig. 4). Surprisingly, in the short term, the combination of CsA and overload triggered a large accumulation of TnIs mRNA after 1 (data not shown) and 2 weeks of this condition (Fig. 3), rather than the anticipated down-regulation of this gene in the presence of this calcineurin inhibitor (4). Moreover, CsA administration to overloaded mice did not appear to influence the expression of MHC mRNAs in

FIG. 2. Administration of calcineurin inhibitors prevents the fast-to-slow fiber conversions associated with overload. Plantaris muscle cross-sections displaying individual fibers labeled (brown) with a type I/slow MHC-specific antibody. Chronic overload induced a progressive increase in the size of all fibers as well as the number of fibers decorated by MHC I antibody after 2 (panel b, slow no. = 24 ± 6) and 4 (panel d, slow no. = 110 ± 32) weeks compared with Sham-operated counterparts (panel a, slow no. = 6 ± 2). Treatment of overloaded mice with CsA effectively counteracted this adaptive response to overload at both 2 (panel c) and 4 (panel e) weeks. Scale bar = 100 μm for panels a, b, and c. Scale bar = 200 μm for panels d and e.

FIG. 3. Transcript levels for MHC and TnI isoforms after 2 weeks of overload-vehicle and overload-CsA treatments. Total RNA obtained from analogous distal portions of Sham-operated (Sham) and overloaded (OV) plantaris muscles treated with vehicle (veh) or CsA after 2 weeks of treatment was reverse-transcribed and amplified using PCR (16). a, ethidium-bromide stained gels of RT-PCR products for MHC I, Ila, IIX, and IIb (left) and TnIs, TnIf, and the 28 S ribosomal RNA subunit (right), generated from cDNA samples pooled within treatment conditions (n = 3 muscles/treatment) for this picture. Negative control lane (i.e., PCR amplification substituting cDNA with water) is marked with a minus. b, abundance of RT-PCR products are expressed relative to Sham-veh levels after 2 weeks of the various treatments. Values represent means ± S.E. counts per minute (cpm) of RT-PCR products derived from individual muscle RNA samples (n = 3 muscles/treatment). Asterisks, a, and b denote significant (p < 0.05) differences from Sham-veh, OV-veh, and OV-CsA treatments, respectively. The 28 S ribosomal RNA served as an internal control and was not different across treatments.

FIG. 4. Transcript levels for MHC and TnI isoforms match muscle phenotype adaptations after 4 weeks of overload-vehicle and overload-CsA treatments. Total RNA obtained from analogous distal portions of the plantaris from Sham-operated (Sham) and 4-week overload-vehicle (OV) mice administered either vehicle (veh) or CsA was reverse-transcribed and amplified using quantitative PCR. Ethidium bromide-stained gels of RT-PCR products for I, Ila, IIX, and IIb MHC, TnIs, TnIf, and the 28 S ribosomal RNA subunit, generated from cDNA samples pooled within treatment conditions (n = 4 muscles/treatment) are shown. Water negative lane is marked with a minus. The 28 S ribosomal RNA subunit served as an internal control and was not different across conditions. Note that plantaris transcript levels from mice administered CsA only were not different from Sham-vehicle counterparts (data not shown).
the short term of this treatment in any way predicted by the protein adaptations observed at this time (Fig. 3). Our data therefore suggest that the influence of calcineurin-NF-AT signaling on the expression of fast and slow isoforms of distinct myofibrillar proteins, including TnIs, in intact animals is more complex than what is proposed to occur in vitro (4) and is not limited to the transcriptional activation of these contractile protein genes.

Our results are the first to show that calcineurin has a profound influence on the accumulation of skeletal muscle contractile proteins under conditions of increased activation. In this sense, the actions of calcineurin in skeletal muscle resemble those recently purported to mediate various forms of hypertrophic cardiac myopathy (2, 3). The etiology of these cardiomyopathies is proposed to involve alterations in the cellular handling of calcium (2, 3), leading to higher sustained intracellular levels of this cation, a condition necessary for calcineurin activation (5). CsA and FK506 can only interfere with the action of calcineurin when this enzyme is in its activated complex than what is proposed to occur.

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REFERENCES

1. Schiaffino, S., and Reggiani, C. (1996) Physiol. Rev. 76, 371–423
2. Molkentin, J. D., Lu, J.-R., Antos, C. L., Markham, B., Richardson, J., Robbins, J., Grant, S. R., and Olsen, E. N. (1998) Cell 93, 215–228
3. Sussman, M. A., Lim, H. W., Gude, N., Taigen, T., Olson, E. N., Robbins, J., Colbert, M. C., Guallart, A., Wieczorek, D. F., and Molkentin, J. D. (1998) Science 281, 1689–1690
4. Chin, E., Olson, E. N., Richardson, J. A., Yang, Q., Humphries, C., Shelton, J. M., Wu, H., Zhu, W., Bassel-Duby, R., and Williams, R. S. (1998) Genes Dev. 12, 2499–2509
5. Dolmetsch, R. E., Lewis, R. S., Goodnow, C. C., and Healy, J. I. (1997) Nature 386, 855–856
6. Hongbo, K., White, E., Gannier, F., Argibay, J. A., and Garnier, D. (1995) Am. J. Physiol. 269, C690–C697
7. Dunn, S. E., and Michel, R. N. (1997) Am. J. Physiol. 273, C371-C383
8. Olha, A. E., Jasmin, B. J., Michel, R. N., and Gardiner, P. F. (1988) J. Neurophysiol. 60, 2138–2151
9. Kandarian, S. C., Peters, D. G., Taylor, J. A., and Williams, J. H. (1994) Am. J. Physiol. 266, C1190-C1194
10. Clipstone, N. A., Fiorentino, D. F., and Crabtree, G. R. (1994) J. Biol. Chem. 269, 26431–26437
11. Dunn, S. E., Burns, J. L., and Michel, R. N. (1999) FASEB J. 13, A84
12. Mene, U., Kagen, A., Cohen, A., Aramburu, J., Schoen, F. J., and Neer, E. J. (1999) Proc. Natl. Acad. Sci. U. S. A. 95, 13893–13898
13. Zhang, W., Kowal, R. C., Rusnak, F., Sikkink, R. A., Olson, E. N., and Victor, R. G. (1999) Circ. Res. 84, 722–7228
14. Michel, R. N., Vu, C. Q., Tetzlaff, W., and Jasmin, B. J. (1994) J. Cell Biol. 127, 1061–1069
15. Phelan, J. N., and Genyean, W. J. (1997) Anat. Rec. 247, 179–188
16. Gauthier, E. R., Madison, S. D., and Michel, R. N. (1997) Pflugers Arch. 433, 664–668
17. Huey, K. A., and Bodine, S. C. (1996) Am. J. Physiol. 271, C2016–C2026
18. Thompson, A. W. (1989) Immuno. Today 10, 6–9
19. Sehgal, S. N. (1995) Ther. Drug Monitor 17, 660–665
20. Dennis, P. B., Fumagalli, S., and Thomas, G. (1999) Curr. Opin. Genet. Dev. 9, 49–54
21. Abbott, R. L., Friday, B. B., Thaloor, D., Murphy, T. J., and Pavlath, G. (1998) Mol. Biol. Cell 9, 2905–2916
22. Nakayama, M., Stauffer, J., Cheng, J., Banerjee-Basu, S., Wawrousek, E., and Buonanno, A. (1996) Mol. Cell. Biol. 16, 2408–2417
23. Crecar, M. M., Hamilton, N. C., Blank, S., Urdea, M. S., and Iannuzzo, C. D. (1989) Mol. Cell. Biochem. 86, 115–123
24. Biring, M. S., Fournier, M., Ross, D. J., and Lewis, M. I. (1998) J. Appl. Physiol. 84, 1967–1975
25. Goy, J.-J., Stauffer, J.-C., Derauz, J.-P., Gillard, D., Kaufmann, U., Kuntzer, T., and Kappenberger, L. (1989) Lancet i, 1446–1447
26. Guerini, D. (1997) Biochem. Biophys. Res. Commun. 234, 271–275