BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **235,** 217–222 (1997)
ARTICLE NO. RC976753

Chronic Stimulation Differentially Modulates Expression of mRNA for Dihydropyridine Receptor Isoforms in Rat Fast Twitch Skeletal Muscle

Yann Péréon, Javier Navarro, Marc Hamilton,* Frank W. Booth,* and Philip Palade
Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77555-0641; and
**Department of Integrative Biology, University of Texas-Health Science Center, Houston, Texas 77225*

Received April 28, 1997

This study examined the effects of low frequency chronic stimulation on expression of the mRNA encoding the two isoforms of the α1 subunit of the dihydropyridine receptor (DHPR) calcium channel, a critical component of skeletal muscle excitation-contraction coupling. RNase protection assay was used to determine alteration in isoform expression in 5-day, 9-day and 13-day chronically stimulated rat *tibialis anterior* muscle, and to compare it with soleus and *extensor digitorum longus* muscles. Low frequency chronic stimulation was associated not only with a significant decrease in the mRNA level of the skeletal isoform of the DHPR, but also with a significant increase in the mRNA level of the cardiac isoform of the DHPR, the overwhelming majority of which was the adult splice variant. Significant levels of cardiac DHPR mRNA expression were also found in normal adult slow twitch soleus muscle. These findings raise the question of a potential role for the cardiac DHPR in certain adult skeletal muscles.

In excitation-contraction (EC) coupling of skeletal muscle, depolarization of the transverse tubular system (TTS) induces a conformational change of the dihydropyridine sensitive calcium channel (DHPR), which is believed to trigger calcium release from the sarcoplasmic reticulum (SR) through the ryanodine receptor (1, 2). DHPRs correspond to the tetrads observed in the TTS (3), and their subunit composition is well known (4): each of the 5 subunits has been cloned and the 170 kDa α1-subunit functions both as an L-type Ca^{2+} channel and as the voltage sensor in skeletal muscle EC coupling (5-8). In heart, where Ca^{2+} entry through the DHPR directly triggers Ca^{2+}-induced Ca^{2+} release from the SR (9, 10), much of the difference from skeletal muscle EC coupling is due to expression of a cardiac-specific isoform of the α1-subunit (11, 12).

Recently, we were surprised to find significant expression of the cardiac isoform of the α1-subunit in diaphragm muscle (13), a skeletal muscle unique in its chronic stimulation at low-frequency. Muscle activity is known to be an important regulatory factor in the expression of different muscle contractile and metabolic properties (14). Low-frequency chronic stimulation is the most widely used model of muscle activity for exploring the relationship between functional changes and alterations at the molecular level in skeletal muscle. It causes a fast-to-slow transformation consisting of profound changes in the expression of proteins involved in the myofibrillar apparatus, calcium regulatory systems and energy metabolism (15), leading to slower contraction and relaxation velocities and more fatigue-resistant behaviour (16).

Both DHPR and ryanodine receptor expression are greatly suppressed in canine skeletal muscle after chronic stimulation for 6-8 weeks (17). In contrast, DHPR expression is markedly increased in rat soleus after biomechanical unloading, which induces a slow-to-fast twitch transformation (18, 19). Both results are consistent with the higher levels of DHPR in fast- vs. slow-twitch muscles (17, 18). However, DHPR levels in both fast- and slow-twitch leg skeletal muscles are increased after run training exercise in rats (20), and they remain unchanged after increased weight bearing by removal of synergist muscles in rats (19).

The purpose of the present study was to assess changes in expression of skeletal and cardiac DHPR α1-subunit mRNAs after up to two weeks of chronic stimulation of fast twitch muscle (tibialis anterior, TA). The cardiac isoforms of some proteins are present in

Abbreviations: DHPR, dihydropyridine receptor; DIA, diaphragm; EC, excitation-contraction; EDL, extensor digitorum longus; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RT-PCR, reverse transcription-polymerase chain reaction; SOL, soleus; SR, sarcoplasmic reticulum; TA, tibialis anterior; TTS, transverse tubule system.

0006-291X/97 $25.00

slow-twitch skeletal muscle fibers (21), and evidence of cardiac DHPR mRNA expression has been reported in developing (22) and regenerating (23) skeletal muscle. In the present study evidence was obtained not only for upregulation of the cardiac isoform of the DHPR in chronically stimulated fast twitch muscle, but also for its expression in normal adult slow twitch muscle.

METHODS

Chronic electrical stimulation. Electrodes and devices were implanted into 12 (200 g) rats for chronic stimulation as described by Mayne *et al* (24). The deep peroneal nerve of the left hindlimb was exposed from the lateral side in anesthetized rats under aseptic conditions. A battery-powered implantable stimulator (25) was implanted in the abdominal cavity, and two stainless steel electrodes were secured ~0.5 cm apart with sutures under the deep peroneal nerve, which supplies motor fibers to the muscle in the anterior compartment of the hindlimb including the tibialis anterior muscle. The same procedure was performed in the right hindlimb except that the electrodes were not connected to a stimulator. Stimulation was begun 2 days later. Rectangular pulses (0.2 ms in duration) were applied continuously at 10 Hz for 24 h/day. Tibialis anterior muscles were dissected from anesthetized rats after 5, 9 and 13 days of stimulation. *Extensor digitorum longus* (EDL), soleus (SOL), *tibialis anterior* (TA) and heart muscles were removed from adult rats (~300 g) heavily anesthetized by pentobarbital sodium (Abbott, Chicago, IL).

RNA isolation. Total cellular RNA was isolated separately from EDL, SOL, control and stimulated TA, and heart muscles using guanidinium isothiocyanate (RNAzol B kit, Biotecx, Houston, TX). Typically, 100 μg of total RNA was obtained from 100 mg of tissue. The ratio of absorbance at 260 nm to that at 280 nm and 28S-to-18S ribosomal RNA ratios were checked.

cDNAs for mRNA analysis. Three cDNAs were used as hybridization probes: 1) a rat 201-bp cDNA (clone 1fb) corresponding to part of the repeat I of the skeletal DHPR α1-subunit, specific for the skeletal muscle isoform (18). 2) a mouse 152-bp cDNA (clone C32) corresponding to the cytoplasmic loop linking repeats II and III of the cardiac isoform of the DHPR α1-subunit. This cDNA was subcloned in the Pst I site of a pBluescript vector from a 928-bp cDNA kindly provided by Dr. N. Chaudhari, specific for the cardiac isoform of the DHPR (22). C32 has the sequence GGGGAaGAgG AtGAAGAgGA GCCaGAGATg CCtGTGgGgC CaCGcCCcCG gCCcCTGtCt GAGCTGCAcC TtAAgGAaAA gGCAGTtCCC ATgCCGGAaG CcAGtgCaTT tTTCATCTTC AGcCCaAaCA ACAgGtTCCG ccTgCaG, where bases in small letters indicate sufficient dissimilarity (26%) between the cardiac and skeletal muscle calcium channels in rabbit (26) to permit selective use in RPA. 3) a rat cDNA probe for GAPDH (Ambion, Austin, TX) was used as an internal marker.

Ribonuclease protection assay (RPA). Antisense RNA probes were produced using T7 polymerase (Stratagene, La Jolla, CA) on linearized C32, 1fb or GAPDH templates in the presence of [α-^{32}P]rUTP (DuPont NEN, Boston, MA). RPAs were carried out using the RPA II Kit (Ambion, Austin, TX), hybridizing 10-30 μg of total RNA from each tissue source to 0.5-1 $\times$ 10^5 c.p.m. of the RNA probe. The RNA was run on a 5% polyacrylamide, 8 M urea gel. Undigested RNA probes were also run on the same gel for use as size markers. However, they have a bigger size than the protected fragments since a portion of the vector upstream of the insert was included. Filters were exposed at $-80°$ on x-ray films (X-OMAT, AR, Eastman Kodak, Rochester, NY) with two intensifying screens for 2 (1fb clone) or 20 hours (C32 clone).

Quantification of mRNA expression. Autoradiograms of RNase Protection assays were subjected to densitometric analysis using the Lynx Densitometer program (Lynx 5000 Digital Imaging Analysis System). The intensity of hybridization of RNAs to the cRNA probes for GAPDH was used to assess possible RNA degradation (none was found). However, it was not used for quantification, as GAPDH expression varies between muscles and may be modified by chronic stimulation (27). Data are expressed as mean values ± SD. The statistical significance of differences in mean values between mRNA levels was assessed by the unpaired *t* test. A $p < 0.05$ level was considered to be significant.

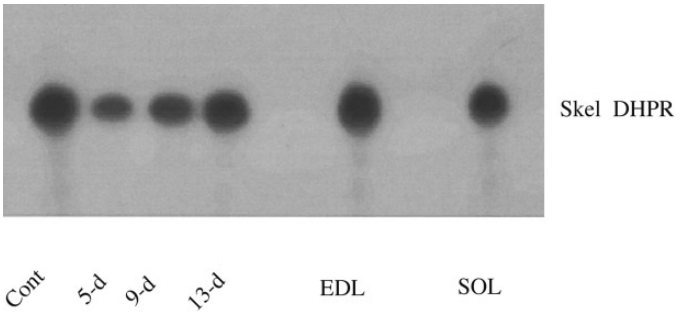

FIG. 1. Expression of mRNA for the skeletal isoform of the DHPR α1-subunit. Representative RNase protection assays demonstrating rapid decrease in 5-day stimulated TA, followed by recovery of expression in 9- and 13-day stimulated muscles, compared with EDL and SOL. 10 μg of total RNA were hybridized with radiolabeled single strand antisense skeletal DHPR RNA probe.

Reverse transcription and polymerase chain reaction (RT-PCR). Expression of the cardiac DHPR α1 subunit was also investigated using RT-PCR. Three primers (GibcoBRL, Gaithersburg, MD) were used: for the cardiac adult splice variant, TACATCCCCAAGAACCAGCA forward and GTGTACCTCGGTGATTGCTA reverse, to yield a 309 bp PCR product (nucleotides 3819-4127, corresponding to the IVS3B region of the rat α1 subunit); for the fetal splice variant the same forward primer was used with a TAGTTTCACTGAGAATGACG reverse primer to yield a 307 bp product. One microgram amounts of total RNA from heart, control and 13-day stimulated muscles were used and RT-PCR was performed with the GeneAmp EZ r*Tth* RNA PCR kit (Perkin Elmer, Roche, Branchburg, NJ), according to the directions of the manufacturer. Samples were amplified in a PTC-150HB Minicycler (MJ Research, Watertown, MA). The RT pre-cycle consisted of a 56°C step for 60 minutes followed by a 94°C step for 2 minutes. The cycling parameters were as follows: 1 min at 94°C, 1 min at 56°C. 40 cycles were performed, followed by a final step at 56°C for 7 min. PCR products were analyzed by electrophoresis of 12.5 μl of each sample on a 2% Metaphor agarose (FMC Bioproducts, Rockland, ME) gel and ethidium bromide staining. Further verification of PCR product identity was determined by size of diagnostic restriction digests.

RESULTS

To assess effects of chronic stimulation of TA muscle on the skeletal isoform of the DHPR-calcium channel mRNA, 10 μg of total RNA from control, 5-day, 9-day and 13-day stimulated TA muscle were hybridized to the 1fb cRNA in RNase protection assays. Skeletal DHPR mRNA expression levels were characterized by an initial dramatic decrease (5 days of stimulation), followed by a progressive recovery (9 and 13 days of stimulation) (figure 1). Quantification of relative expression showed that after 5 days of stimulation, the

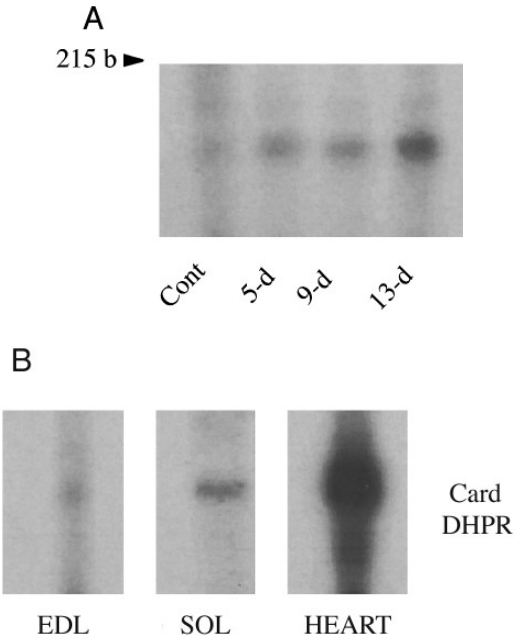

FIG. 2. Expression of mRNA for the cardiac isoform of the DHPR α1-subunit. Representative RNase protection assays demonstrating presence of the expected 152-bp protected fragment in chronically stimulated (5-d, 9-d, 13-d) rat TA skeletal muscle, compared with control TA, EDL, SOL and heart (H) muscles. 20 μg of total RNA were hybridized with radiolabeled single strand antisense cardiac DHPR RNA probe.

skeletal isoform of the α1 subunit exhibited less than half the expression of the control; after 13 days of stimulation, expression was still lower than in control (77.4 $\pm$ 11.5 %, n = 3, $p < 0.05$ between control and 15-days) (figure 3A). Single specific bands were found for all skeletal muscle samples (fig 1), and there was no detectable signal with rat heart RNA (not shown). The expression of mRNA encoding the skeletal isoform of the DHPR was higher in EDL than in SOL (figure 1). Similar results were obtained in at least 3 separate experiments (figure 2A). The intensity of the hybridization from SOL represented 65.2 $\pm$ 14 % of the level of expression in EDL ($p < 0.05$).

When total RNA from stimulated TA muscle was hybridized to the C32 cRNA, a consistent progressive increase of the expression of the cardiac isoform of the α1 subunit gene was observed (figure 2 & 3B), attaining 12.4 $\pm$ 2.6 % of the expression level in the heart (n = 3, $p < 0.01$ between control and 13-days). The cardiac DHPR α1 subunit cDNA probe hybridized to cardiac total RNA yielding a single specific band, and a weak signal was also observed with EDL and SOL RNA (figure 2; see also Péréon *et al.* (23)). For precise quantification, we repeated the experiments using 25-30 μg of total RNA of EDL, SOL and heart muscle, and reproducible results were obtained in all 4 separate sets of experiments. The standardized intensity of the hybridization signal was 2.5 $\pm$ 1.4 % and 7.9 $\pm$ 0.6 % of the level of heart expression, in EDL and SOL, respectively (figure 3B) ($p < 0.05$ between EDL and SOL).

In the same sets of experiments, GAPDH cRNA probe was used together with DHPR probes. There was a discrete but generally consistent decrease of the GAPDH gene expression level with chronic stimulation, and expression was higher in heart and EDL than in SOL (not shown). For these reasons, quantification of DHPR message levels was normalized to total RNA rather than GAPDH expression. Normalization with

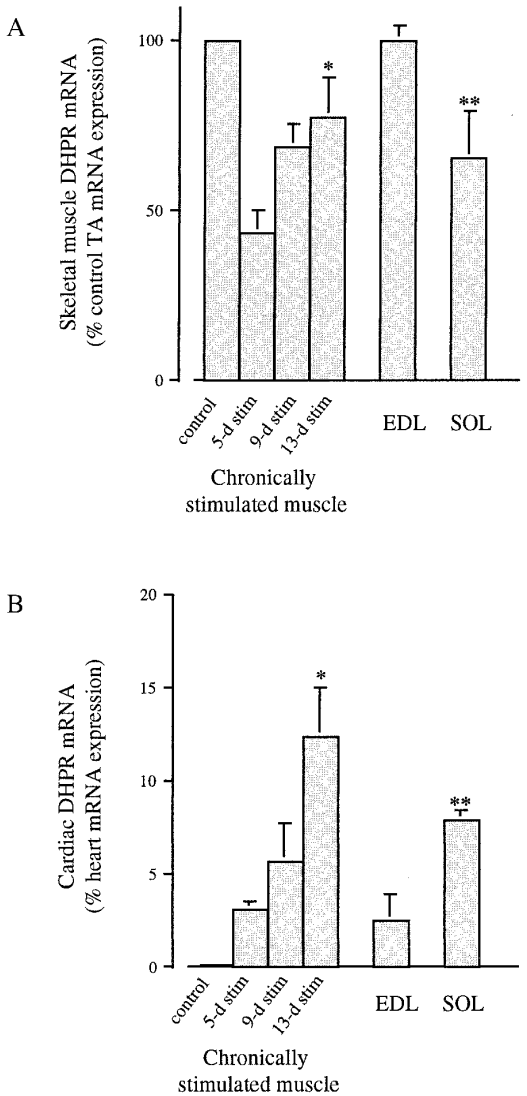

FIG. 3. Relative mRNA expression levels of the skeletal (A) and cardiac (B) isoforms of the DHPR-calcium channel in control and chronically stimulated (5-d, 9-d, 13-d) rat TA, and in rat EDL and SOL. Quantification of autoradiograms was performed by densitometry, according to the amount of total RNA. Data were expressed as percentage of control TA expression (skeletal isoform) or rat heart expression (cardiac isoform). Error bars represent SD. *$p < 0.01$ for control *vs* 13-day stimulated TA (n = 3).

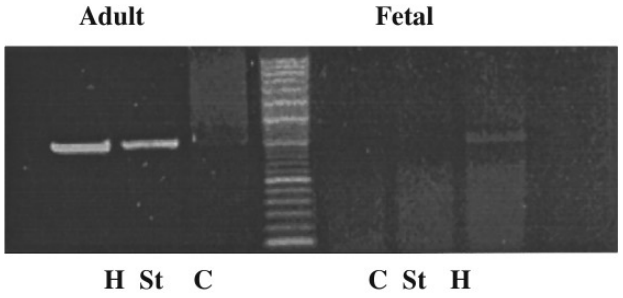

FIG. 4. Detection of cardiac α1 subunit splice variant transcripts by RT-PCR in heart (H), 13-day stimulated tibialis anterior (St) and contralateral control TA (C). Representative ethidium bromide staining of the RT-PCR products electrophoresed on a 2 % agarose gel. The left three lanes display products obtained with primers for the adult splice variant (309 bp product); the right three lanes products obtained with primers for the fetal splice variant (307 bp product). In between are 20 bp and 100 bp ladder markers starting at 100 bp.

respect to GAPDH would have indicated a decrease in skeletal DHPR mRNA of 51.3 % at 5 d; and for cardiac DHPR mRNA an increase to 9.8 % in TA after 13 d stimulation, and levels in EDL and SOL of 1.5 % and 6.8 %, in all cases relative to that in heart.

RT-PCR detected the adult splice variant of the cardiac DHPR mRNA in both heart and 13-day stimulated TA muscle. No significant signal was obtained in control muscle with the experimental conditions used (figure 4). RT-PCR detected the fetal splice variant in heart only but not in either control or stimulated TA muscle.

DISCUSSION

We observed a decrease in skeletal DHPR mRNA and an increase in cardiac DHPR mRNA after chronic stimulation of the TA. Previous work revealed that both the DHPR and the ryanodine receptor, which each play a critical role in excitation-contraction coupling, are greatly reduced in 6-to-8-week stimulated canine skeletal muscle (17), but the differential DHPR isoform expression was not evaluated in that study. In the present work we also report here for the first time the expression of the cardiac DHPR α1 subunit mRNA in normal slow twitch muscle.

Skeletal isoform of the DHPR. The number of DHPRs is higher in fast-twitch than slow-twitch muscle (28), as observed here at the mRNA level in EDL and SOL muscles. In conditioned fast-twitch muscle we found that chronic stimulation of TA induces a dramatic decrease in the expression of the skeletal DHPR after 5 days of stimulation, when skeletal DHPR mRNA levels were decreased by more than half. The levels increased gradually over the next several days, but remained significantly lower than those of control muscle. A similar biphasic change in a mitochondrial enzyme has been reported in response to chronic stimulation (27). The transcriptional regulation of skeletal DHPR and certain other proteins may be under some additional local control, distinct from that involved in a simple fast-to-slow twitch muscle transformation. Another possible explanation of the initial decrease could be that chronic stimulation induces degeneration of fast-twitch fibers which are secondarily replaced by newly formed slow-twitch fibers, as reported in rabbit (29). In that case, immature fibers are likely to express a lower level of skeletal DHPR (23, 30). However, chronic low frequency stimulation in rat skeletal muscle slowly caused a fiber transformation but no degeneration-regeneration, and no increase in slow type I fibers was observed after two weeks of stimulation (31). Since the amount of transverse tubule in SOL is half that in EDL, and feet density/area of junctional transverse tubules is the same in the two muscles (32), it is also possible that chronic stimulation induces a decrease of transverse tubule surface area (17), but morphological studies of such muscle are still needed.

Cardiac isoform of the DHPR. Using a highly sensitive RNase protection assay, we demonstrate that SOL significantly expresses the mRNA encoding the cardiac isoform of the DHPR, whereas it is barely detectable in EDL, and moreover that chronic stimulation of TA induces the appearance of an even higher level of mRNA for the cardiac isoform. The failure of Mejia-Alvarez *et al.* (33) to detect the cardiac DHPR isoform in adult rat skeletal muscle using RT-PCR might have been the result of poor PCR optimization or of using fast twitch skeletal muscle RNA which was partially degraded. From our RT-PCR results we conclude that the cardiac isoform is composed nearly exclusively of the adult splice variant form. While the PCR product obtained with the fetal primers was less intense in the heart positive control, this reflects the lower mRNA levels found in adult heart (34). It should be emphasized that this is a study at the RNA level, and we have no information regarding the expression of the protein in these chronically stimulated muscles. RNA isolation was performed from the whole muscle, and it could be proposed that fibroblasts (35) or vascular smooth muscle cells (36) could be responsible for the expression of the cardiac DHPR mRNA. However, fibrosis has not been described as a histological feature in stimulated rat muscle (31) and increased vascularization associated with the ~50 % increase of the capillary network (15) could not account for the far larger increases in cardiac DHPR mRNA levels observed here. Moreover, we have detected the cardiac isoform of the DHPR on adult rat fibers immunohistochemically in other studies (13). Thus, it is likely that skeletal muscle fibers are the cells which express this mRNA.

As mentioned earlier, a regeneration process, which has been shown to induce transient expression of the

cardiac DHPR mRNA in rat skeletal muscle (23), does not occur in chronically stimulated rat muscle (31) and therefore cannot account for the increased expression seen here. Even though cardiac DHPR mRNA is also expressed in SOL, a slow twitch muscle, it is probably the chronic stimulation, rather than the fiber type transformation it induces, which is responsible for its increased expression in stimulated TA: rat EDL stimulated 12d at the same frequency exhibits no alteration of fiber composition, and rat TA should transform even more slowly (31). In a separate study, we found that rat and mouse diaphragm muscle express this isoform at a similar level as SOL (13). Diaphragm is an interesting model of skeletal muscle undergoing chronic activity, but still mainly composed of fast-twitch fibers (37). Thus, the type of fibers prone to express the cardiac DHPR mRNA in skeletal muscle remains unclear.

Although no studies have been carried out on chronically stimulated muscle, skeletal muscle EC coupling in general is not acutely dependent on external calcium. While rat SOL has been described to exhibit twitches whose amplitude decreases in lowered extracellular calcium or in the presence of calcium channel blockers (38, 39), its ability to generate maximal intracellular calcium release at +30 mV (40), a potential which should elicit diminished calcium entry, argues that EC coupling in SOL is more skeletal-like than cardiac-like. This should not be surprising in view of the far greater expression of the skeletal isoform of the DHPR. However, it has been been suggested that, during periods of sustained muscle activity, influx of extracellular calcium may be involved in modulation of SR calcium release (4) or in replenishment of intracellular reserves (41). Increased cardiac DHPR expression could also contribute to maintenance of tetanic responses or to fatigue resistance. The skeletal isoform of the DHPR would result in negligible Ca^{2+} entry during an action potential because of the slow activation kinetics of the channel, whereas the faster activating cardiac isoform could allow significant entry (12).

In summary, chronic stimulation affects components of EC coupling in a more significant manner than previously shown: not only does it decrease the expression of the skeletal DHPR, but it also significantly increases the expression of the cardiac isoform of the DHPR $\alpha 1$ subunit in skeletal muscle. Comparison with EDL and SOL muscles (present study), or with DIA (13), which contracts more-or-less continuously like the heart, may yield clues to its possible physiological significance, which remains to be determined.

ACKNOWLEDGMENTS

We are grateful to Dr. Nirupa Chaudhari for mouse cardiac DHPR cDNA. This work was supported by NIH AR 44208 (F.B.). Dr. Yann Péréon was supported by grants from the Ministère des Affaires Etrangères (Paris, France, Programme Lavoisier), the Philippe Foundation (New York, NY), and the Association de Formation Continue des Praticiens Hospitaliers (Nantes, France). This work is part of the Ph.D. thesis of Y.P.

REFERENCES

1. Rios, E., Pizarro, G., and Stefani, E. (1992) *Ann. Rev. Physiol.* **54,** 109–133.
2. Melzer, W., Herrmann-Frank, A., and Luttgau, H. C. (1995) *Biochim. Biophys. Acta* **1241,** 59–116.
3. Franzini-Armstrong, C., and Jorgensen, A. O. (1994) *Ann. Rev. Physiol.* **56,** 509–534.
4. Catterall, W. A. (1991) *Cell* **64,** 871–874.
5. Beam, K. G., Adams, B. A., Niidome, T., Numa, S., and Tanabe, T. (1992) *Nature* **360,** 169–171.
6. Perez-Reyes, E., Kim, H. S., Lacerda, A. E., Horne, W., Wei, X. Y., Rampe, D., Campbell, K. P., Brown, A. M., and Birnbaumer, L. (1989) *Nature* **340,** 233–236.
7. Tanabe, T., Beam, K. G., Powell, J. A., and Numa, S. (1988) *Nature* **336,** 134–139.
8. Tanabe, T., Takeshima, H., Mikami, A., Flockerzi, V., Takahashi, H., Kangawa, A., Kojima, M., Matsuo, H., Hirose, T., and Numa, S. (1987) *Nature* **328,** 313–328.
9. Callewaert, G. (1992) *Cardiovasc. Res.* **26,** 923–932.
10. Näbauer, M., Callewaert, G., Cleeman, L., and Morad, M. (1989) *Science* **244,** 800–803.
11. Tanabe, T., Beam, K. G., Adams, B. A., Niidome, T., and Numa, S. (1990) *Nature* **346,** 567–569.
12. Tanabe, T., Mikami, A., Numa, S., and Beam, K. G. (1990) *Nature* **344,** 451–453.
13. Péréon, Y., Navarro, J., and Palade, P. T. (1997) *Biophys. J.* **72,** A149.
14. Booth, F. W., and Tseng, B. S. (1993) *News Physiol. Sci.* **8,** 165–169.
15. Pette, D., and Vrbova, G. (1992) *Rev. Physiol. Biochem. Pharmacol.* **120,** 115–202.
16. Dux, L. (1993) *Rev. Physiol. Biochem. Pharmacol.* **122,** 69–147.
17. Ohlendieck, K., Briggs, N., Lee, K. F., Wechsler, A. W., and Campbell, K. P. (1991) *Eur. J. Biochem.* **202,** 139–147.
18. Kandarian, S., O'Brien, S., Thomas, K., Schulte, L., and Navarro, J. (1992) *J. Appl. Physiol.* **72,** 2510–2514.
19. Kandarian, S. C., Peters, D. G., Favero, T. G., Ward, C. W., and Williams, J. H. (1996) *Am. J. Physiol.* **39,** C1588–C1594.
20. Saborido, A., Molano, F., Moro, G., and Megias, A. (1995) *Pflügers Archiv.* **429,** 364–369.
21. Jorgensen, A. O., Arnold, W., Pepper, D. R., Kahl, S. D., Mandel, F., and Campbell, K. P. (1988) *Cell Motil. Cytoskeleton* **9,** 164–174.
22. Chaudhari, N., and Beam, K. G. (1993) *Dev. Biol.* **155,** 507–515.
23. Péréon, Y., Navarro, J., Sorrentino, V., Louboutin, J. P., Noireaud, J., and Palade, P. T. (1997) *Pflügers Archiv.* **433,** 221–229.
24. Mayne, C. N., Mokrusch, T., Jarvis, J. C., Gilroy, S. J., and Salmons, S. (1993) *FEBS Lett.* **327,** 297–300.
25. Salmons, S., and Jarvis, J. C. (1991) *Med. Biol. Eng. Comput.* **29,** 554–556.
26. Mikami, A., Imoto, K., Tanabe, T., Niidome, T., Mori, Y., Takeshima, H., Narumiya, S., and Numa, S. (1989) *Nature* **340,** 230–233.
27. Cresci, S., Wright, L. D., Spratt, J. A., Briggs, F. N., and Kelly, D. P. (1996) *Am. J. Physiol.* **39,** C1413–C1420.
28. Jorgensen, A. O., Shen, A. C., Arnold, W., Leung, A. T., and Campbell, K. P. (1989) *J. Cell Biol.* **109,** 135–147.

29. Maier, A., Gambke, B., and Pette, D. (1986) *Cell Tissue Res.* **244,** 635–643.
30. Kyselovic, J., Leddy, J. J., Ray, A., Wigle, J., and Tuana, B. S. (1994) *J. Biol. Chem.* **269,** 21770–21777.
31. Delp, M. D., and Pette, D. (1994) *Cell Tissue Res.* **277,** 363–371.
32. Appelt, D., Buenviaje, B., Champ, C., and Franzini-Armstrong, C. (1989) *Tissue Cell* **21,** 783–794.
33. Mejia-Alvarez, R., Tomaselli, G. F., and Marban, E. (1994) *J. Physiol. (Lond.)* **478,** 315–329.
34. Gidh-Jain, M., Huang, B., Jain, P., Battula, V., and El-Sherif, N. (1995) *Biochem. Biophys. Res. Commun.* **216,** 892–897.
35. Soldatov, N. M. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 4628–4632.
36. Koch, W. J., Ellinor, P. T., and Schwartz, A. (1990) *J. Biol. Chem.* **265,** 17786–17791.
37. Louboutin, J. P., Fichter-Gagnepain, V., Thaon, E., and Fardeau, M. (1993) *Neuromusc. Disord.* **3,** 463–469.
38. Kotsias, B. A., Muchnik, S., and Obejero-Paz, C. A. (1986) *Am. J. Physiol.* **250,** C40–C46.
39. Gallant, E. M., and Goettl, V. M. (1985) *Eur. J. Pharmacol.* **117,** 259–265.
40. Delbono, O., and Meissner, G. (1996) *J. Membrane Biol.* **151,** 123–130.
41. Almers, W., McCleskey, E. W., and Palade, P. T. (1985) Calcium in Biological Systems, (Rubin, R. P., and Weiss, G. B., Eds.), pp. 321–330, Plenum, New York.