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TPA Has No Influence on the Expression of Myosin Heavy Chain Isoforms in Cultured Adult Cardiac Muscle Cells

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Abstract The effect of a tumor promoter, 12-0-tetradecanoyl phorbol-13-acetate (TPA), on the expression of myosin heavy chain isoforms in cultured rat cardiac ventricular muscle cells was studied. The previous preliminary report [Claycomb WC (1988): "Biology of Isolated Adult Cardiac Myocytes." In Clark WA, Decker RS, Borg TK (eds): New York: Elsevier, pp 284–287] indicated that TPA turns off the expression of myosin heavy chain genes in cultured adult cardiac myocytes. Electrophoretic and immunocytochemical analyses were carried out in the present studies. The myosin heavy chain isoform profiles of cardiac myocytes exposed to TPA at concentrations of 50–250 ng/ml culture medium for varying periods were similar to those of controls that were grown in the absence of TPA, showing predominant isoform V1. Immunofluorescence microscopy with monoclonal antibodies to cardiac ventricular isomyosin revealed the structural organization of myosin in TPA-treated cells. The organization of myosin was variable among different myocytes and within a single myocyte. Immunofluorescence microscopy was extended to the examination of the organization of α-actinin which did not differ from that of myosin in some myocytes. In contrast to the previous report [Claycomb, 1988], this study has demonstrated that TPA has no influence on the expression of myosin heavy chain isoforms in cultured adult ventricular cardiac muscle cells. © 1992 Wiley-Liss, Inc.

Key words: adult cardiac myocytes, cell culture, TPA, myosin heavy chain isoforms, anti-myosin, anti-α-actinin

The tumor promotor TPA has a reversible influence on the disassembly of myofibrils, the contractile apparatus of muscle cells. This influence is particularly predominant in maturing skeletal muscle cells [Cohen et al., 1977; Croop et al., 1980; Lin et al., 1987, 1989] where TPA reversibly stops spontaneous contractions. An immunoreactive myofibrillar contractile protein, myosin is found to be completely eliminated from these skeletal muscle cells after 60–72 h of TPA exposure [Lin et al., 1989]. Choi et al. [1991] reported that phorbol esters reversibly decreased the mRNA levels of myosin heavy chain, myosin light chain ⅓, myosin light chain 2, cardiac and skeletal α-actin, and skeletal troponin T genes in chick skeletal myotubes. These decreases were attributed at least in part to the decreases in transcription rates. Some of the decreases in mRNA levels of cardiac and skeletal α-actin genes were the result of increased mRNA turnover. These investigators noted that the steady state message level of cardiac troponin T did not change upon exposure to phorbol esters. The transcription rate of this gene decreased only transiently. Recently, Zhu et al. [1991] investigated the effect of TPA on the transcriptional activity and mRNA stability of four contractile protein genes including skeletal and cardiac α-actin, cardiac troponin C, and myosin light chain 1f, and two nonmuscle genes, β-cytoplasmic actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), in terminally differentiated cultured myotubes. They found that the transcriptional activity of the α-cardiac actin and cardiac troponin C genes decreased by 8 h after exposure to TPA, whereas other muscle and nonmuscle genes showed no change. While carrying out pulse-chase experiments of in vivo labeled RNA, these investigators observed significant reductions in mRNA half-lifes for all the contractile protein mRNAs investigated. However, the half-lifes of β-actin and GAPDH mRNA did not change. Working with the cultured adult cardiac muscle cells treated with TPA, Moses and Claycomb [1989] reported that myofibrils of treated cells became disorganized in a manner similar to that observed in skeletal muscle cells by the above investigators [Cohen et al., 1977;

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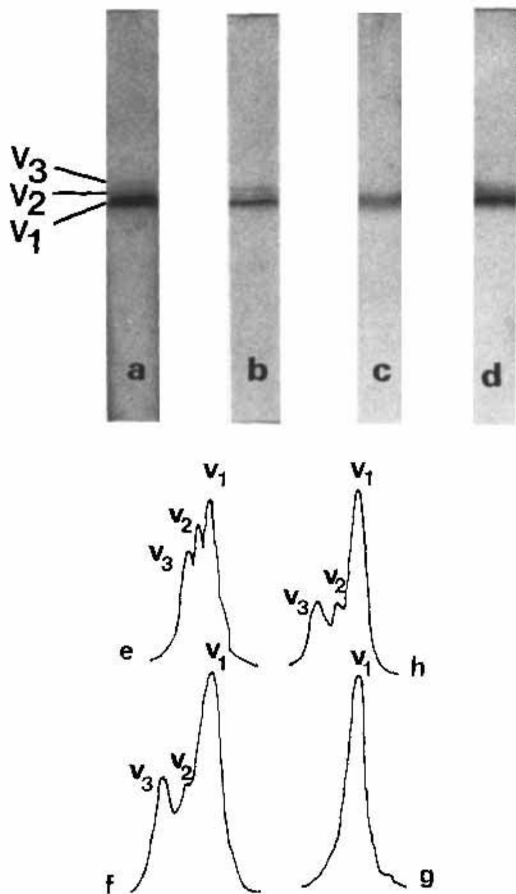


Fig. 1. Electrophoretic determinations of adult ventricular myosin, myosin of cultured adult cardiac ventricular muscle cells grown in the presence of TPA or in the absence of TPA. **a:** Rat ventricle 250 g. **b:** Adult rat cardiac muscle cells in culture without TPA. **c:** Adult cardiac muscle cells cultured with TPA at a concentration of 50 ng/ml medium for 5 days (day 7 of culture to day 12). **d:** Adult cardiac muscle cells cultured with TPA at the same concentration as in c for 2 weeks (day 7 of culture to day 21). **e:** Absorbance profiles of myosin isoform bands of 250 g adult rat ventricle. **f:** Control culture. **g:** Culture treated with TPA (50 ng/ml medium) for 5 days. **h:** Culture treated with TPA (50 ng/ml medium) for 2 weeks.

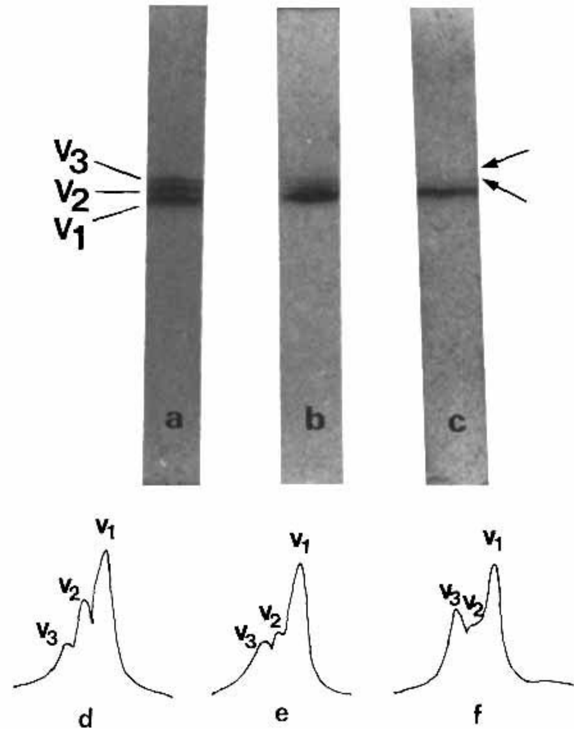


Fig. 2. Electrophoretic analyses of myosin of cultured adult rat cardiac ventricular muscle cells grown in the presence of TPA or in the absence of TPA. **a:** Adult cardiac muscle cells in culture without TPA. **b:** Adult cardiac muscle cells cultured with TPA at a concentration of 250 ng/ml medium for 5 days (day 7 of culture to day 12). **c:** Adult cardiac muscle cells cultured with TPA at the same concentration as in b for 2 weeks (day 7 of culture to day 21). Arrows show faint V_2 and V_3 bands. **d:** Absorbance profiles of myosin isoform bands of control culture. **e:** Culture exposed to TPA (250 ng/ml medium) for 5 days. **f:** Culture treated with TPA (250 ng/ml medium) for 2 weeks.

Croop et al., 1980; Lin et al., 1987, 1989]. Claycomb [1988] also reported that TPA shuts down the expression of muscle-specific myosin heavy chain genes in cultured adult cardiac muscle cells.

In contrast to skeletal muscle cells, spontaneous contractions of cells were observed in embryonic and adult cardiac muscle cells exposed to TPA [Lin et al., 1989; Nag and Lee, 1990]. Recent studies [Nag and Lee, 1990] on the organization of myofibrils in cultured adult cardiac muscle cells treated with TPA demonstrated that responsiveness of cardiac myocytes to TPA not only differs from that of skeletal muscle cells, but also varies within a population of cardiac myocytes. Having obtained these results, we investigated the expression of myosin heavy chain isoforms and the immunoreactive myosin heavy chain phenotype in cultured adult cardiac muscle cells. Contrary to Claycomb's [1988] results, we observed that TPA has no effect on the expression of myosin heavy chain isoforms. Furthermore, myosin is not eliminated from TPA-treated myocytes, and the phenotypic pattern of immunoreactive myosin varies extensively among TPA-exposed cardiac myocytes. This study focuses mainly on the response of myosin in cultured adult cardiac myocytes exposed to TPA.

TABLE I. Myosin Isoform Contents of Adult Rat Ventricles, and Cultured Adult Rat Ventricular Cardiac Myocytes Grown in the Presence or Absence of TPA*

	Percentage of myosin isoform in total myosin, cell culture			
	Medium with TPA		Medium without TPA	Adult
Isoform	5 d	2 wk	(12 d/3 wk)	ventricle
V1	100 (± 9)	93 (± 8)	95 (± 10)	60 (± 6)
V2				28 (± 4)
V3		9 (± 2)	5 (± 0.7)	12 (± 3)

*Isoforms were determined by graphic resolution of absorbance peaks for protein and measuring the area under the peaks. Values are means ± SD for 12 determinations. Isoform V2 content was measured only for the adult ventricle. The determinations were based on experiments where TPA was used at a concentration of 50 ng/ml medium. The longer durations of control cultures reflect the addition of the initial week prior to the exposure of cultures to TPA.

MATERIALS AND METHODS

Cell Culture

Following the methods of our previous studies [Nag and Cheng, 1981; Nag et al., 1983, 1990a], adult rat ventricular cardiac muscle cells were isolated from male and female rats weighing approximately 200–250 g. The experiments were carried out following the experimental design of Claycomb [1988]. Since we did not observe any effect of TPA on the expression of myosin heavy chain isoforms with 50 ng/ml culture medium as used by Claycomb [1988], we also investigated the effect of higher doses of TPA, such as 100 ng/ml medium and 250 ng/ml medium, on the expression of myosin isoforms in cultured adult cardiac myocytes. Cardiac myocytes were exposed to TPA for 5 days in Claycomb's [1988] design. In addition, we exposed cardiac myocytes to TPA for 2 weeks to examine whether TPA has any effect on the expression of myosin isoforms during the extended period of exposure. Cytosine arabinoside was included in the culture medium for the first 7 days of culture to eliminate non-muscle cell contamination. Three sets of cell cultures were run, using three different doses of TPA (Sigma Chemical Co., St. Louis, MO; Chemsyn Science Lab., Lenexa, KS) as indicated above. The control culture was run without TPA in the culture medium as carried out by Claycomb [1988] and the culture media of both control and experimentals were changed every other day, using fresh medium with or without TPA. Following the studies of Lin et al. [1989], we conducted additional experiments in which media were changed every day so that myosin isoforms expression could be compared to that in the experiments in which media were changed every other day. We observed no difference in results between the two types of experiments and consequently we fed our cultures every other day. The cells were examined routinely with phase optics of a Zeiss Axiovert 35 inverted epifluorescence microscope for observation of the external cellular morphology and contractility of myocytes. The cardiac myocytes were processed for gel electrophoresis at intervals of 5 and 14 days of exposure to TPA and for immunofluorescence microscopy at intervals of 1, 2, 5, 10, and 14 days of exposure to TPA. Since there was no difference in the expression of myosin isoforms between treatments of three different doses of TPA, gel pictures with lowest and highest doses of TPA are included in the text.

Myosin Extraction and Electrophoresis

Cardiac muscle cells were collected from culture dishes, scraping the cells with a plastic cell scraper (GIBCO, NY). Myosin extraction and native-gel electrophoresis were carried out, following our previous methods [Nag and Cheng, 1984, 1986; Nag et al., 1990b]. Gels were scanned on the LKB-2202 ultrascan laser densitometer for densitometric tracings and quantitation of the area under the protein isoform peaks.

Immunofluorescence Microscopy

Cultures were rinsed with PBS and fixed in 2% formaldehyde (freshly prepared from paraformaldehyde) in PBS for 10 min, followed by rinses in PBS. Cultures were incubated in 0.1 M glycine in PBS for 15 min. The cells were then permeabilized using 0.1% Triton X-100 in PBS for 10 min, followed by rinses in PBS and incubation in 0.5% BSA to block nonspecific binding of primary antibodies. The monoclonal antibodies (CCM-52 and RCM-42) against cardiac ventricular isomyosin were the generous gift of Drs. R. Zak and W.A. Clark, University of Chicago and Northwestern University (Chicago, IL). The specificities of these antibodies were described previously [Clark et al., 1982; Nag et al., 1985] and dilutions were 1:50. Anti-α-actinin antibody

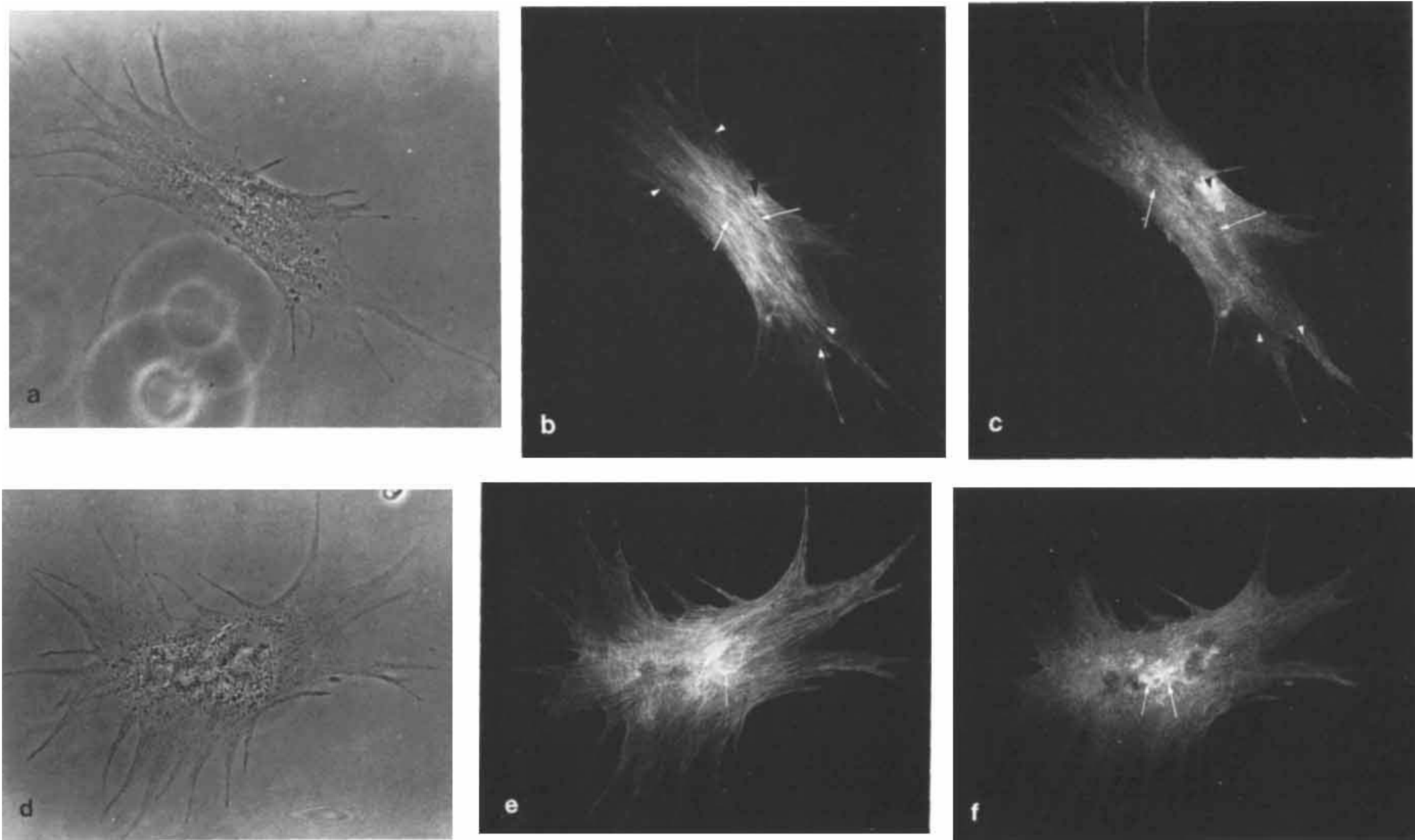


Figure 3.

was purchased from transformation Research Inc. (Framingham, MA). The specificity of this antibody was described previously [Isobe et al., 1988] and the dilution was 1:30. After the primary antibody (CCM-52, RCM-42, and rabbit anti-α-actinin), incubation at 37°C humidified atmosphere for 1 h, cells were washed three times with PBS, incubated with secondary antibodies (FITC-labeled goat anti-mouse IgG and Rhodamine-labeled goat anti-rat IgG, FITC-labeled goat anti-rabbit IgG; ICN Biomedicals, Inc., Costa Mesa, CA) in the manner described for the primary antibodies incubation, washed, and mounted using aqua-poly/mount (Polysciences, Inc., Warrington, PA). Cells were examined with a Zeiss epifluorescence microscope using excitation filters for fluorescein and rhodamine.

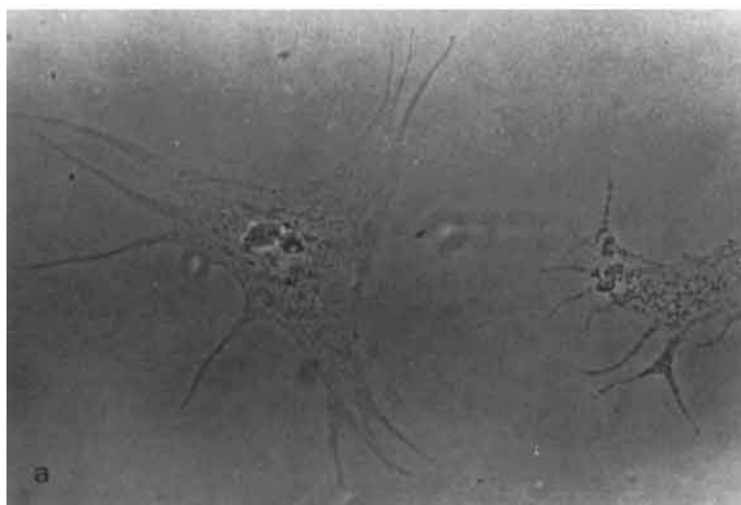
RESULTS

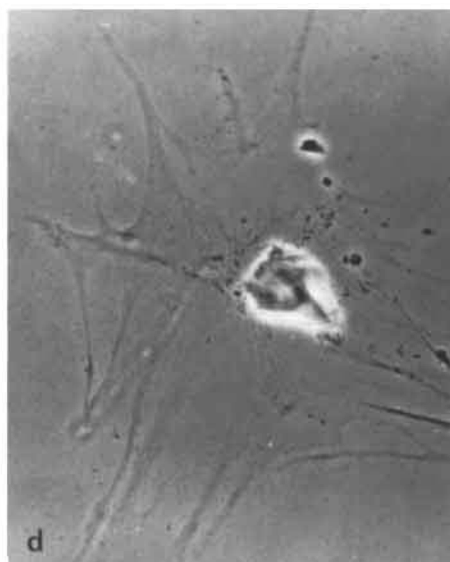
Electrophoretic Pattern of Native Myosin

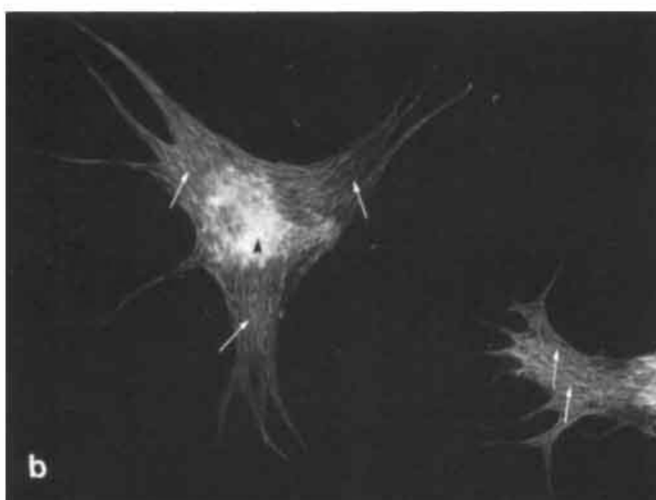
Adult rat cardiac myocytes in vitro with or without TPA. The electrophoretic profiles of myosin extracted from three different sets of culture of adult rat ventricular cardiac myocytes exposed to three different concentrations of TPA (50 ng/ml medium, 100 ng/ml medium, 250 ng/ml medium) for varying periods of time were similar to those of the controls, which were grown in the absence of TPA. The myosin of control and experimental myocytes that were exposed to TPA from days 1–14 of culture contained a predominant isoform band which was comparable to the V1 band of the ventricle of adult rats (Figs. 1, 2). Isoform bands V2 and V3 often were faint or absent in cultured experimental and control cells. It is known that the adult rat cardiac myocytes in culture sometimes exhibit only the predominant myosin isoform band and do not show minor bands [Nag and Cheng, 1986; Nag et al., 1990b] as was also observed in in vivo young rat ventricles [Hoh et al., 1977]. The presence of a single predominant isoform band in Figure 1c,g merely shows the usual expression of myosin isoforms in cultured adult rat myocytes as indicated above and does not indicate the influence of TPA on the expression of myosin isoform profiles. The expression of myosin isoform profile in 7-day cultures prior to the exposure to TPA was similar to that of the control showing the predominant isoform V1 as was reported in previous studies *without using* any drug [Nag and Cheng, 1986]. Figures 1e–h and 2d–f show the absorbance profiles of myosin of the control and experimental cardiac myocytes along with the in vivo ventricular myosin. Myosin from all these sources contained the predominant peak absorbance of isoform V1, while the peak absorbencies of V2 and V3 were lower than that of isoform V1 or absent as indicated above. Myosin isoform contents of in vivo ventricular, control, and experimental cardiac myocytes were determined by graphic resolution of absorbance peaks for protein and by measurement of the area under the peaks. The results, summarized in Table I, showed no overall difference in myosin isoform contents between the control myocytes and the TPA-treated myocytes which contained predominant isoform V1.

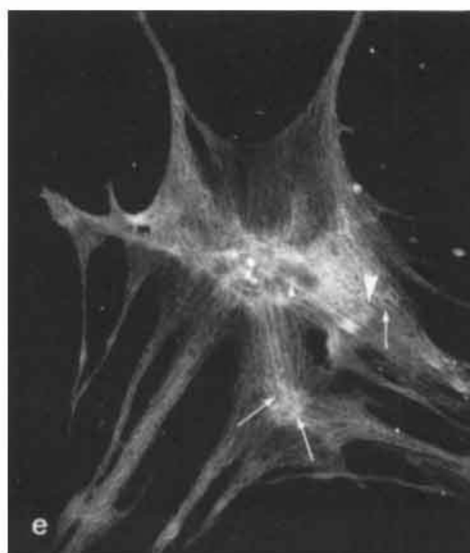
Organization of myosin and α-actinin in TPA-treated myocytes. Immunofluorescence microscopy has been used to examine the structural organization of myosin in cardiac myocytes exposed to TPA. Similar results were obtained using each of the two antibodies to cardiac ventricular isomyosin. The structural organization of myosin was observed to be more variable

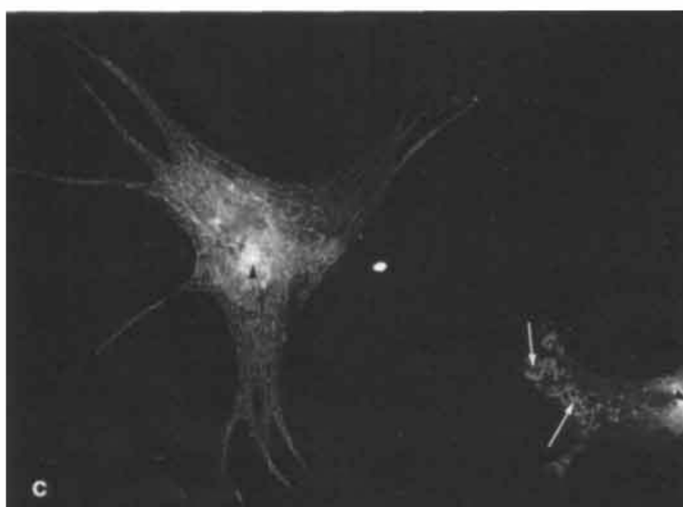
Fig. 3. Immunofluorescent micrographs of cultured adult rat ventricular cardiac myocyte treated with TPA (50 ng/ml medium) and stained doubly with a monoclonal antimyosin antibody (CCM-52) and a polyclonal anti-α-actinin antibody. **a:** Phase-contrast micrograph. **b:** A myocyte exposed to TPA for 5 days (day 7 of culture to day 12) and stained with antimyosin antibody. Note the presence of striated myofibrils exhibiting myosin periodicities in the central part of the cell body (arrows). Myosin-positive unstriated fibrils are seen to be organized in the terminal parts of the cell (arrowheads). The black arrowhead that shows amorphous myosin corresponds with its counterpart in panel c showing amorphous α-actinin. **c:** Myocyte stained with anti-α-actinin antibody shows periodicities in the central region of the cell (arrows). Anti-α-actinin antibody positive unstriated fibrils are visible at either extremity of the cell body (arrowheads). **d–f:** Immunofluorescent micrographs of control adult rat ventricular cardiac myocytes grown in the absence of TPA and stained doubly with antimyosin antibody (CCM-52) and a polyclonal anti-α-actinin antibody. **d:** Phase-contrast micrograph. **e:** A control myocyte cultured for 12 days and stained with antimyosin antibody. Myosin-positive striated vicinity of the cell, while fibrils are distributed in the middle and its myosin-positive most unstriated fibrils are running in the cytoplasmic processes of the cell. Note the presence of amorphous myosin near the central part of the cell (arrows). **f:** Myocyte stained with anti-α-actinin antibody exhibits striations in the cell body with the exceptions of cytoplasmic processes, most of which contain unstriated fibrils. Note the presence of amorphous α-actinin in the central region of the cell (arrows). ×500.











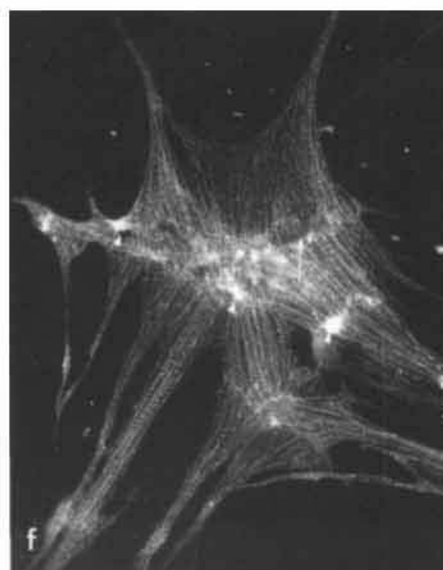


Figure 4.

among different myocytes and within a single myocyte. Some TPA-treated myocytes contained a moderate number of myofibrils in approximately the central part of the cell (Fig. 3b). These myofibrils exhibited myosin periodicities comprising A-bands that measured approximately 1.6 μm, as did those in the controls (Fig. 3e). The terminal parts of these cells contained unstriated myosin-positive fibrils that extended into the cytoplasmic processes of cells (Fig. 3b,e). A number of TPA-treated myocytes had an amorphous form of myosin in approximately the middle portion of the cell. Surrounding this central amorphous mass of myosin were unstriated myosin-positive fibrils that were organized along the different axes of the cell, terminating into the cytoplasmic processes of cells (Fig. 4b). In addition to these structures, regular or irregular aggregates of myosin were observed in these cells as compared to their control counterparts, which contained strings of punctate myosin that were distributed at different axes of the cells (Figs. 4e, 5b). A considerable number of TPA-treated and control myocytes contained abundant myosin-striated fibrils along with some myosin-positive unstriated fibrils (Figs. 5e, 6b).

Fig. 4. Cultured adult cardiac myocytes treated with TPA (50 ng/ml medium) for 2 weeks (day 7 of culture to day 21) and stained doubly with antimyosin (CCM-52) and anti-α-actinin antibodies. **a:** Phase-contrast micrograph. **b:** Amorphous form of myosin is localized in the central part of the cell (arrowhead), while the myosin-positive unstriated fibrils diverge towards the cytoplasmic process of the cell (arrows). Note the absence of striated myofibrils. A part of an adjacent myocyte is present in the lower-right corner, containing myosin-positive unstriated fibrils (arrows). **c:** Myocytes stained with anti-α-actinin antibody exhibit centrally located amorphous α-actinin (arrowhead) and some irregular aggregates of α-actinin, which also are seen in the cytoplasmic processes of the cell together with unstriated fibrils. Note that the adjacent cell exhibits centrally located amorphous α-actinin (arrowhead) together with vesicular α-actinin in other parts of the cell body (arrow). **d–f:** Experimental cultured adult cardiac myocyte grown in the presence of TPA (50 ng/ml medium) for 5 days (day 7 of culture to day 12) and stained doubly with antimyosin and anti-α-actinin antibodies. d: Phase-contrast micrograph. **e:** Myosin is organized mainly in unstriated fibrillar form distributed throughout the body of the myocyte. Regular and irregular aggregates of myosin are organized linearly or irregularly (arrows). Rare myosin-striations are present (large arrowhead). Amorphous myosin are shown by small arrowheads. **f:** α-actinin are primarily organized in strings of punctate structures throughout the body of the cell. Irregular patches of amorphous α-actinin are visible in different parts of the cell (arrowheads). The myocyte shows some α-actinin striations (arrow). ×500.

Immunofluorescent studies were extended to the organization of another crucial myofibrillar protein, α-actinin, which constitutes a major component of the Z-line of the myofibril. Double-staining of the same cell with anti-myosin and anti-α-actinin revealed that the overall organization of α-actinin in some TPA-treated cardiac myocytes did not differ from that of myosin (Fig. 3b,c). Periodicities of α-actinin were observed in the central myofibrillar region of the cell, with unstriated anti-α-actinin positive fibrils in the terminal parts of the cell. Some of the TPA-treated myocytes that contained a centrally located amorphous form of myosin contained amorphous α-actinin together with some irregular aggregates of α-actinin that extended to the cytoplasmic processes of the cell (Fig. 4b,c). Surrounding this central amorphous α-actinin were anti-α-actinin–positive unstriated fibrils that extended to the cytoplasmic processes of the cell along with the irregular aggregates of α-actinin described above. In addition, some myocytes exhibited some vesicular forms of α-actinin (Fig. 4c).

The organization of α-actinin in a number of doubly stained myocytes did not always follow the organization of myosin. In these myocytes α-actinin was present in various forms, exhibiting linearly organized punctate structures, striations, amorphous mass, vesicles, and unstriated fibrils that sometimes formed a reticulum around a centrally located amorphous myosin (Figs. 4f, 5c,f), and did not match with the organization of myosin in the same cells (Figs. 4e, 5b,e). A considerable number of myocytes contained abundant α-actinin–striated fibrils (Fig. 6c) as observed in myosin (Fig. 6b). The cytoplasmic processes of these cells contained mainly α-actinin–positive unstriated fibrils, punctate α-actinin, and sparse amorphous α-actinin.

DISCUSSION

It is well established that the myosin isoform profile of normal adult rats consists of three isoforms, among which isoform V1 is predominant over isoforms V2 and V3 [Hoh et al., 1977; Lompre et al., 1981; Clark et al., 1982; Nag and Cheng, 1984, 1986]. Sometimes a single band of predominant isomyosin was observed in the gel [Hoh et al., 1977; Nag and Cheng, 1984, 1986; Nag et al., 1990b]. These three myosins (V1, V2, V3) are made of two types of myosin heavy

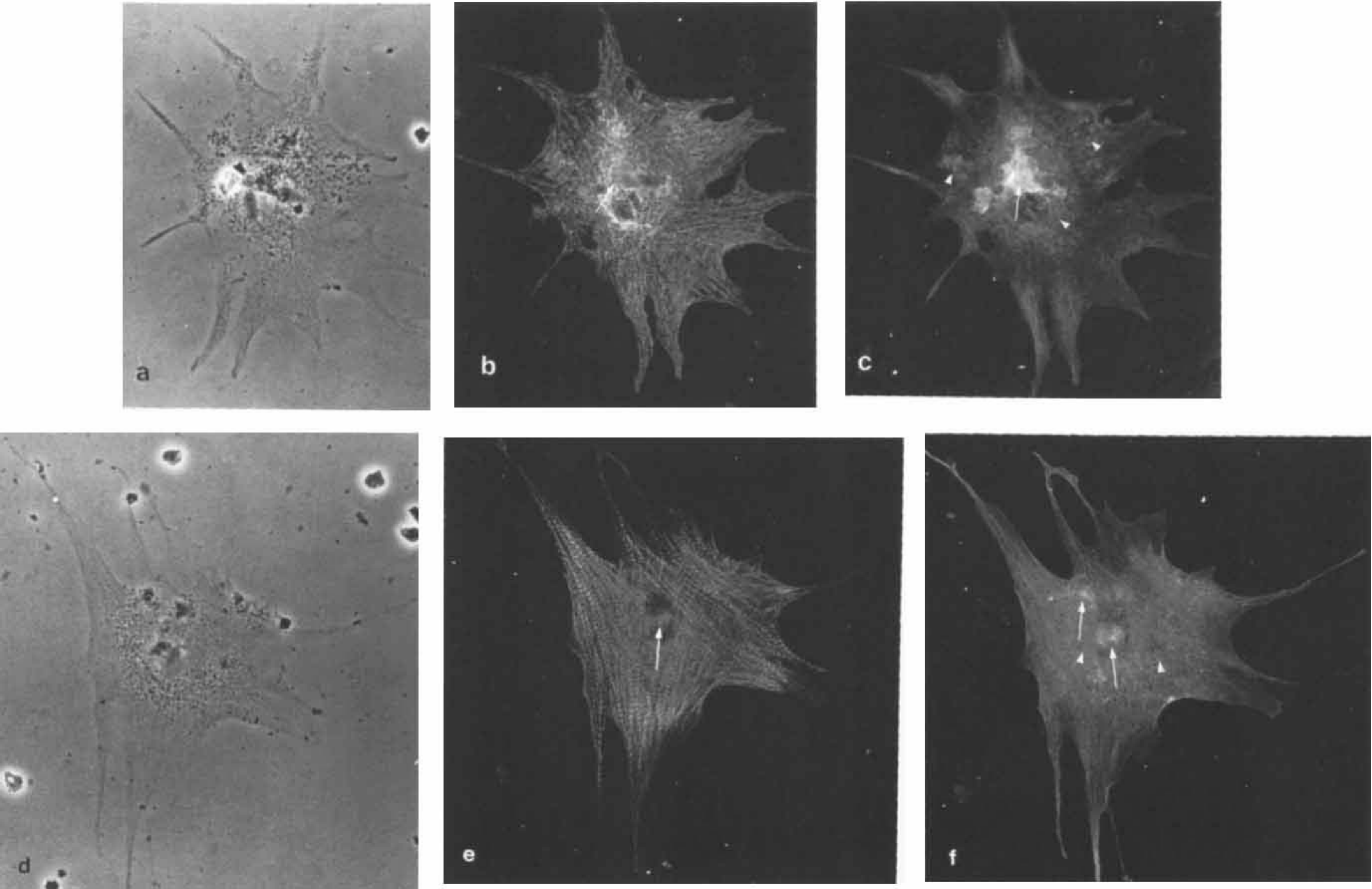


Figure 5.

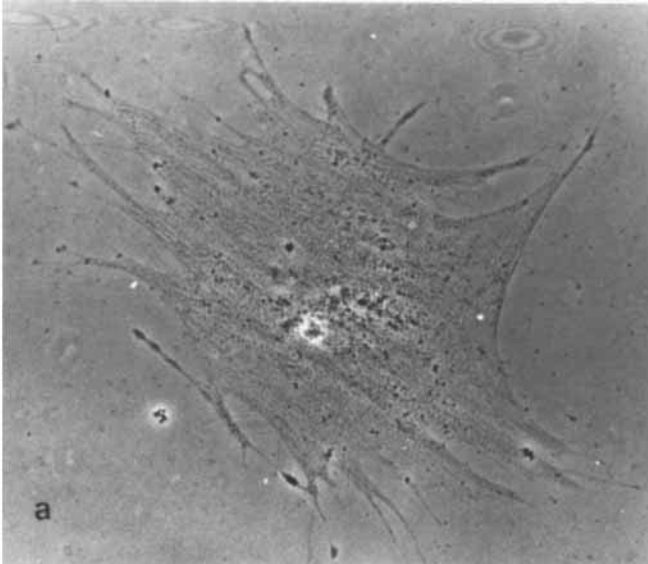
chains, referred to as α and β. V1 and V3 are made of αα and ββ homodimers, respectively, while V2 is an αβ heterodimer [Hoh et al., 1979; Chizzonite et al., 1982]. These two types of myosin heavy chains are found to be the products of two different genes that have been isolated and characterized [Mahdavi et al., 1982, 1984; Sinha et al., 1982]. The ratio of the different cardiac isomyosins is developmentally regulated [Hoh et al., 1977; Sartore et al., 1981; Clark et al., 1982]. The distributions of these isomyosins undergo changes in certain pathological and experimental conditions such as mechanical overload [Lompre et al., 1979; Mercadier et al., 1981; Gorza et al., 1981; Litten et al., 1982; Sheuer et al., 1982], diabetes [Dilman, 1980; Malhotra et al., 1981], and changes in thyroid hormone levels [Hoh et al., 1977; Chizzomite et al., 1982; Sartore et al., 1981; Sinha et al., 1982; Nag and Cheng, 1984, 1986; Lompre et al., 1984; Gustafson et al., 1987; Mahdavi et al., 1989].

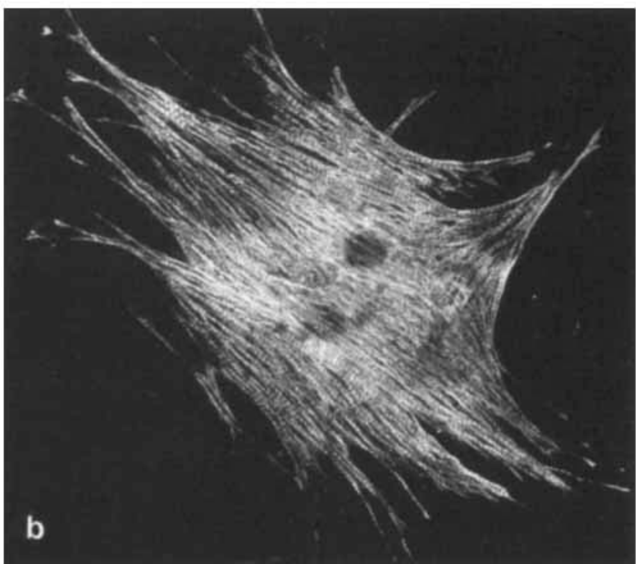
Recently Claycomb [1988] reported that TPA turns off myosin heavy chain gene in cultured adult cardiac muscle cells that have been exposed to TPA (50 ng/ml). This study involved the isolation of the total cellular RNA and its hybridization with the radioactive DNA probe of myosin heavy chain gene. We carried out studies on the effect of TPA on the expression of myosin heavy chain isoforms in cultured adult cardiac muscle cells and observed no effect of TPA on the expression of myosin heavy chain isoforms. It is evident from the electrophoretic analyses of myosin that the expression of myosin heavy chain isoform is not eliminated from the myocytes treated with TPA in contrast to the report of Claycomb [1988]. Initially, we used the same concentration of TPA (50 ng/ml medium) as used by Claycomb [1988] and did not observe any cessation of expression of myosin heavy chain. Subsequently, we tested increased doses of TPA, such as 100 ng/ml culture medium and 250 ng/ml culture medium, yet no effect of TPA on the expression of myosin heavy chain isoforms was observed. The present findings agree with those of previous observations [Lin et al., 1989; Nag and Lee, 1990] that reported the contractility of embryonic and adult cardiac myocytes, respectively, even after the exposure to TPA. The contractility of skeletal and cardiac muscle cells require the participation of myosin heavy chain, which evidently is present in the TPA-treated contractile cardiac muscle cells. The expression of myosin heavy chain isoforms and their phenotypic pattern in TPA-treated myocytes also have been investigated, using monoclonal antibodies to cardiac ventricular myosin heavy chain isoforms.

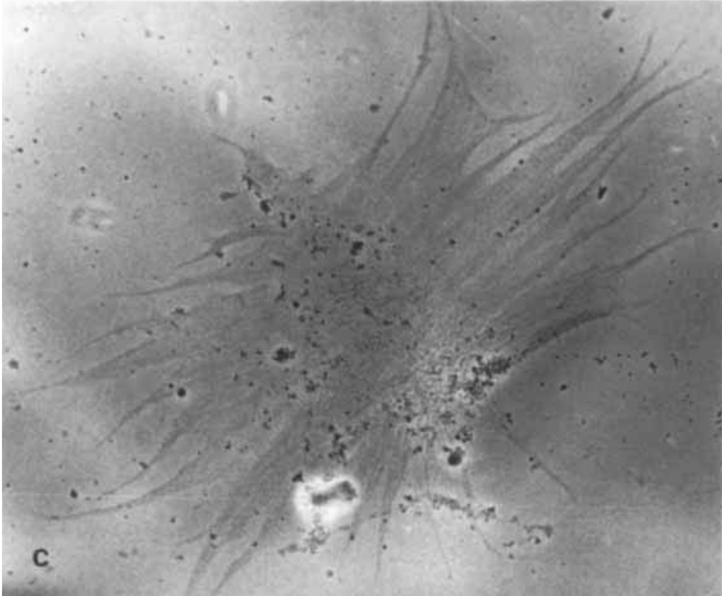
Myosin heavy chain positive A-bands and other structures, such as unstriated fibrils, amorphous and vesicular myosin, and irregular myosin aggregates, along with the result of electrophoretic analyses of native myosin, demonstrate clearly that these cells are capable of expressing myosin heavy chain isoforms after exposure to TPA at a concentration of 50 ng/ml medium or higher doses of TPA. In addition, when we extended these studies to the organization of α-actinin, a major component of the Z-line of the myofibril, we observed that the expression of α-actinin was not turned off in TPA-treated cultured cardiac myocytes. This finding also agrees with the observations of the contractility of TPA-treated cardiac myocytes discussed above, indicating that the Z-line is involved in the contractility of the TPA-treated skeletal and cardiac muscle cells.

Although the present studies followed the same experimental design, including controls (Materials and Methods), as Claycomb [1988], the difference in results between these two stud-

Fig. 5. Control adult cardiac myocytes grown in the absence of TPA for 12 days and stained doubly with antimyosin and anti-α-actinin antibodies. **a:** Phase-contrast micrograph. **b:** Myosin is organized mainly in the form of strings of punctate structures running at different axes of the cell. The terminal parts of the cytoplasmic processes of the cell exhibit mainly unstriated fibrils. Amorphous myosin is seen in the central part of the cell (arrow). **c:** An irregular mass of amorphous α-actinin is visible in the middle part of the cell (arrow). Vesicular forms of α-actinin are present in considerable amounts (arrowheads). Myosin in unstriated fibrils and amorphous form exists in different parts of the cell, including cytoplasmic processes. **d–f:** Cultured control adult cardiac myocytes grown in the absence of TPA for 3 weeks and stained doubly with antimyosin antibody (CCM-52) and anti-α-actinin antibody. **d:** Phase-contrast micrograph. **e:** Note the abundance of myosin-striated fibrils in the cell body with unstriated fibrils distributed in the terminal parts of the cytoplasmic processes of the cell. Centrally located amorphous myosin is shown by an arrow. **f:** Myocytes stained with anti-α-actinin show centrally located amorphous form of α-actinin along with some scattered amorphous α-actinin in the cell body (arrows). Surrounding the central amorphous α-actinin is a reticulum of unstriated α-actinin fibrils (arrowheads). α-actinin–positive unstriated fibrils outside the reticulum are seen to run in the cytoplasmic processes of the cell. ×500.







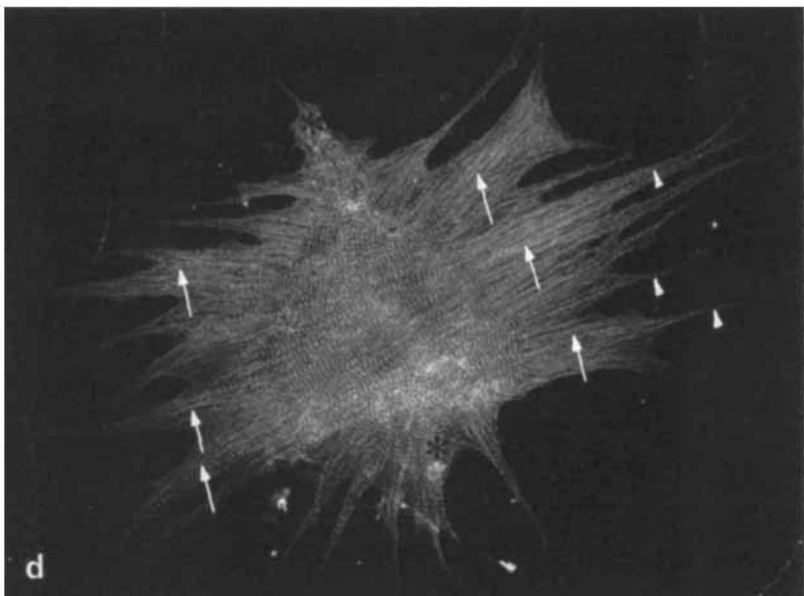


Figure 6.

Fig. 6. Immunofluorescent micrograph of a cultured adult rat ventricular cardiac myocyte treated with TPA at a concentration of 50 ng/ml medium for 5 days (day 7 of culture to day 12) and stained singly with antimyosin antibody (CCM-52). **a:** Phase-contrast micrograph. **b:** Note the abundance of myosin-positive striated myofibrils in the cell. Some unstriated fibrils are present. A small amount of amorphous myosin is visible in tips of certain cytoplasmic processes of the cell. **c,d:** Cultured adult rat ventricular cardiac myocyte exposed to TPA with a concentration of 50 ng/ml medium for 5 days (day 7 of culture to day 12) and stained singly with anti-α-actinin antibody. c: Phase-contrast micrograph. d: The cell exhibits plenty of α-actinin periodicities along with α-actinin–positive unstriated fibrils that run along the most cytoplasmic processes of the cell (arrows). Punctate (arrowheads) and amorphous (asterisks) forms of α-actinin are visible in the cell. ×500.

ies as discussed may be due to the fact that the response of cardiac muscle cells to TPA is more complex than previously believed, thus requiring more research to understand this phenomenon. The contractile proteins of myofibrils in skeletal muscle cells are more affected than those in cardiac muscle cells, showing reversible degradation and elimination of these proteins in skeletal muscle cells. Although the activation of TPA in cells involves its binding to protein kinase C, the influences of TPA on the metabolic pathways responsible for the regulation of expression and maintenance of myofibrillar contractile proteins presumably differ between skeletal and cardiac muscle cells. Also, the different responses of skeletal and cardiac muscle cells to TPA may depend on the cellular receptors for TPA, which possibly differ in number and in physiological activity between these two types of muscle cells.

ACKNOWLEDGMENTS

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