**β-Adrenergic Agonists Stimulate the Synthesis of Noncontractile but Not Contractile Proteins in Cultured Myocytes Isolated From Adult Rat Heart**

Isabelle Dubus, Jane-Lyse Samuel, Françoise Marotte, Claude Delcayre, and Lydie Rappaport

The effect of catecholamines on adult myocardial protein synthetic activity was studied by use of an experimental model of isolated adult rat cardiac myocytes maintained in culture for 1–6 days. During this period, the majority of myocytes retained their rod-shaped morphology, but the cell number decreased progressively (50% of the initial density after 2 days in culture). Between day 1 and day 3 in culture, the specific synthetic activities of total proteins and of electrophoretically purified myosin heavy chain and actin ([35S]phenylalanine incorporation into protein, in disintegrations per minute per microgram protein) decreased (−19%, −32%, and −73%, respectively). Addition of isoproterenol or norepinephrine (10 nM) from the onset of the culture for 3 days increased the specific activity of both total and noncontractile proteins (>20%) but had no effect on the specific activity of myosin heavy chain and actin when compared with 3-day cultured control cells. β-Adrenergic receptors are specifically required to mediate this increase in total protein synthesis. This finding was demonstrated by the inhibitory effects of propranolol; neither prazosin nor yohimbine showed any effect. The pattern of synthesized protein during adrenergic stimulation was qualitatively evaluated by use of [35S]methionine incorporation and gel electrophoresis. The general pattern of labeled proteins did not differ significantly from that of control cells; this occurrence suggests that isoproterenol harmoniously stimulates the synthesis of noncontractile proteins. These findings demonstrate that low doses of β-adrenergic agonists have an anabolic effect on adult cardiac quiescent myocytes that do not affect the major contractile proteins. Regulation of myofibrillar protein synthesis may be more dependent on myocyte contractile activity. (Circulation Research 1990;66:867–874)

The development of cardiac hypertrophy is triggered by numerous factors including increased wall stress and adrenergic hormones (for a review, see Reference 2). The interpretation of in vitro experiments is often limited by complicated systemic interactions. In vitro models of cell culture permit the direct analysis of effects of a single variable at the myocyte level. Using this approach, Simpson3,4 demonstrated that α1-adrenergic stimulation by itself promotes hypertrophy of neonatal rat myocytes in culture and concluded that α1-adrenergic stimulation might be a primary stimulus for cardiac hypertrophy.4 On the other hand, Cooper et al,5 using cultures of adult feline cardiac myocytes, did not observe significant myocyte hypertrophy in response to norepinephrine, an α-β-adrenergic agonist, and concluded that in adult mammalian cells the primary determinant of hypertrophy is mechanical.5,6 The discrepancy between these two studies could result from a difference in the animal developmental stage. Indeed, in adult mammals, the sensitivity to α1-adrenergic stimulation and to the α1-receptor number is decreased.7,8 Moreover, the cardiac responsiveness to β-adrenergic stimulation in mammals is greater in adults than in neonates,9 and in vivo chronic isoproterenol (a β-adrenergic agonist) treatment promotes rat cardiac hypertrophy.10 The absence of an in vitro effect of norepinephrine on adult feline myocyte growth through β-adrenergic

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receptors could also arise from either species specificity or culture conditions. Finally, it is possible that stimulation of growth in adult myocytes is more dependent on the inotropic and chronotropic effects of an adrenergic stimulation than in neonatal cells.

To obtain a better understanding of the respective roles of adrenergic stimulation and mechanical work on the induction of adult rat cardiac hypertrophy, we have developed a model of adult rat cardiac myocytes maintained quiescent in culture. The consequences of the myocyte culture for up to 6 days on the protein synthetic activity were investigated first. Then the responses of myocytes to both isoproterenol and norepinephrine were analyzed with the aid of specific α- or β-adrenergic antagonists. Metabolic effects of adrenergic agonists on myocytes were analyzed quantitatively by measuring the incorporation of [14C]phenylalanine into total protein, myosin heavy chains (MHCs), and actin and qualitatively by separating proteins labeled with [35S]methionine by use of polyacrylamide gel electrophoresis (PAGE). We show that β-adrenergic stimulation of adult rat myocytes, kept quiescent in culture, induces a harmonious increase in the synthesis of noncontractile proteins but that the synthesis of the major myofibrillar proteins is insensitive to this hormonal stimulation.

Materials and Methods

Cardiocyte Isolation

Adult male Wistar rats weighing 280–320 g (Iffa-Credo, Lyon, France) were used in all experiments. Calcium-tolerant myocytes were isolated by cardiac retrograde aortic perfusion, as previously described; the entire procedure was performed under sterile conditions. Cell cultures were then prepared by the method of Eckel et al with minor modifications. Briefly, freshly isolated cells were incubated in the presence of 1 mM calcium at 37°C for 10 minutes and then washed with culture medium (BM86 Wissler, Boehringer Ingelheim Pharmaceuticals, Ridgefield, Connecticut) containing 4% fetal calf serum (Boehringer Ingelheim Pharmaceuticals), 100 IU/ml penicillin, and 0.1 μg/ml streptomycin (M+FCS). The number of cells and the percentage of rod-shaped myocytes were determined in a Malassez chamber. Cardiocytes were then plated on 35-mm Petri dishes (Falcon, Becton Dickinson, Lincoln Park, New Jersey) (1.5×10^7 rod-shaped cells/dish, in 1 ml M+FCS) preincubated for at least 3 hours with M+FCS. Attachment of the rod-shaped cells to the substrate was favored by incubating the dishes at 37°C in a humidified atmosphere consisting of 5% CO₂, 16% O₂, and 79% N₂ for 3 hours. After this period, the M+FCS was removed. A new serum-free medium (BM86 Wissler, Boehringer Ingelheim Pharmaceuticals) containing antibiotics and 10⁻⁹ mol/l insulin was used for all culture procedures and renewed every 24 hours.

Viability of the cells was assessed by their ability to attach to the substrate. Thus, the cultures were examined daily with an inverted light microscope to check the cell density and the percentage of rod-shaped cells. In contrast to neonatal cells, which contract spontaneously, adult myocytes do not show significant contraction during the first two days in culture. Therefore, a more specific assessment of cardiocyte integrity was made from the contractile response of cells to electrical stimulation at 0.5 Hz for 15 minutes at 37°C. At least one dish from each experiment was tested.

Experimental Protocol

Adrenergic agents, isoproterenol (Winthrop-Breon Laboratories, New York, New York) and norepinephrine (Sigma Chemical, St. Louis, Missouri), were freshly prepared for each experiment as 1 mM stock solutions that were serially diluted in BM86 medium, containing sufficient ascorbic acid (0.2 mM) to inhibit catecholamine oxidation.

For assessing the effects of catecholamines on cardiocytes, the initial conditions were those described for the control group. After the attachment period, adrenergic agents were added at a final concentration of 1 nM–10 μM; dilutions were freshly prepared for each media change during the period studied. Propranolol (10 μM) (Sigma Chemical) was used as a β-adrenergic antagonist. In adult cardiac myocytes, prazosin (Pfizer Laboratories, New York, New York), an α₁-adrenergic antagonist, was used at a concentration of 0.1 μM, which is sufficient to block the α₁-receptors in adult rat myocytes. Higher concentrations of prazosin such as 10⁻² M (data not shown) were toxic, as previously shown in the neonate. Yohimbine, an α₂-adrenergic antagonist (Sigma Chemical), was also used at a concentration of 0.1 μM.

Estimation of the Rate of Total Protein Synthesis

Protein synthesis in cultured cells, plus or minus catecholamines, was determined by monitoring the incorporation of L-[14C]phenylalanine ([14C]phe) (specific activity, 450 mCi/mmol; NEN, dupont de Nemours, Wilmington, Delaware) into the total thioracetic acid (TCA)-precipitable cell proteins according to Eckel et al. [14C]phe (1 μCi/ml/dish) was added to the culture medium for the indicated times (0–24 hours). At the end of the incubation period, the medium was removed, and the dishes were rinsed three times with cold HEPES buffer containing (mM) NaCl 130, KCl 4.8, KH₂PO₄ 1.2, glucose 5, and HEPES 25, pH 7.4, equilibrated with oxygen. The proteins were precipitated by the addition of 10% TCA at +4°C for 60 minutes. The precipitates were scraped free with a rubber policeman, transferred into test tubes, washed two times with TCA, and then dissolved in 1N NaOH. Aliquots were used for scintillation counting and protein determination.
Myocytes were incubated for 24 hours with [14C]phe starting at either day 0 or 48 hours after plating, as described above. Proteins from total extracts were separated by preparative electrophoresis (10% PAGE). After staining with 0.3 M CuCl2, MHC and actin bands were excised from each gel. In two experiments, the entire gel area between MHC and actin was also analyzed. The gel slices were destained with 0.25 M EDTA/0.25 M Tris-HCl, pH 9.2, and the proteins were electroeluted in a dialysis apparatus (Bio-Rad Laboratories, Cambridge, Massachusetts). The eluted proteins were then precipitated with 25% TCA, washed two times in 10% TCA, and dissolved in 1N NaOH. This fraction was used for protein determination and scintillation counting. Estimation of the specific activity for each contractile protein or noncontractile protein set was given by the ratio of disintegrations per minute [14C]phe incorporated per micrograms protein.

Measurement of Protein Synthesis for Actin and Myosin Heavy Chain

Cells were incubated at 37°C for 2 hours in 1 ml culture medium containing 65 μCi/ml [35S]methionine (NEN, dupont de Nemours). Incubations were stopped by rinsing dishes three times with ice-cold HEPES buffer. Cells were then lysed in 100 μl buffer containing 0.3% sodium dodecyl sulfate (Merck, Darmstadt, FRG), 1% β-mercaptoethanol, 0.1% DNase (Sigma Chemical), 0.2% RNase (Sigma Chemical), and 0.1 mM phenylmethylsulfonyl fluoride (Sigma Chemical). The lysate was scraped from the culture dish, frozen in liquid nitrogen, and stored at −70°C until needed. Equal amounts of either protein or radioactivity were loaded onto either 10% or 10–20% gradient polyacrylamide gels and resolved by electrophoresis. Dried gels were then subjected to autoradiography. The autoradiograms of gels loaded with the same amount of radioactivity were scanned by a PH16 Vernon densitometer (Paris, France) to evaluate the radioactivity incorporated into each band. Results were normalized on the basis of the amount of protein per dish or alternatively on the total acid-precipitable radioactivity.

Results

Viability and Protein Synthetic Capacity of Adult Cardiac Cells in Culture

The method used for the isolation of adult rat cardiac myocytes enabled us to obtain a reproducible yield of Ca2+-tolerant rod-shaped myocytes (3–6×106 cells/heart) corresponding to 72% of the total myocyte number at the end of the purification step. After the attachment period and washings, the percentage of rod-shaped cells increased to 92%. During the culture period, nearly all myocytes were quiescent with very few cells exhibiting a spontaneous contractile activity when observed by light microscopy, as previously described. During the entire period in culture, those cells attached to the substrate were found to be living based on their ability to contract synchronously with electrical stimulation. During the first 3 days in culture, the changes in both the cell density and the percentage of rod-shaped cells were similar to those previously described for the same period. By day 5, the cell density had dramatically decreased, and the percentage of rod-shaped myocytes also decreased but not to the same extent (Table 1). Round myocytes undergo a dedifferentiation-redifferentiation process with time of culture (for a review, see Reference 17).

Protein synthesis was estimated by measuring the incorporation of [14C]phe into acid-precipitable proteins since this amino acid has been shown to satisfy
all the criteria for measurements of protein synthesis in cardiac muscle tissue and cells.\textsuperscript{4,15,23} Incubations with \textsuperscript{14}C]phe incorporation were continued for 24 hours since 3–4-hour incubation periods were too short to show significant alterations in the total protein synthesis activity of cultured cells\textsuperscript{15} (Figure 1). During this period, the rate of \textsuperscript{14}C]phe incorporation was found to be linear for each day analyzed (Figure 1). Between days 1 and 2 in culture, the rate of total protein synthesis decreased (–17%). Extending the culture period to 6 days resulted only in a slight further decline (–8%) in the basal incorporation rate (Table 1). In culture, rod-shaped cells that become rounded (column 2 of Table 1) have probably maintained their capacity to synthesize proteins as assessed by the relatively constant rate of \textsuperscript{14}C]phe incorporation into total proteins during this period (column 3 of Table 1).

The measured specific activity of the electrophoretically purified MHCs and actin after a 24-hour period of \textsuperscript{14}C]phe incorporation demonstrated that the synthetic activity of contractile proteins in quiescent adult myocytes decreased. One day after plating, values (as disintegrations per minute \textsuperscript{14}C]phe per microgram protein) were 56±6 for MHCs and 168±15 for actin 1 day after plating (n=3), which decreased, respectively, to 38±5 and 44±1.4 at day 3 (n=5).

Changes in cultured myocyte protein synthetic activity were estimated qualitatively by \textsuperscript{35}S]methionine labeling and PAGE analysis after loading the same amount of radioactivity on each lane (Figure 2). The intensity of the \textsuperscript{35}S labeling for the different peptides, particularly that of the contractile proteins (kDa: MHC 200, actin 45, tropomyosin 35, myosin light chain 1 25, and myosin light chain 2 20), clearly decreased as early as day 1; the labeling intensity of other peptides either remained constant or increased (kDa: 240, 62, 54, 32, and 23, respectively). At day 3, fluorogram analysis indicated a relative decrease in the labeling of both MHC and actin. Values (expressed as the percent of total \textsuperscript{35}S]methionine label in the gel lane) were 17.9% for MHC and 13.2% for actin by day 0 (n=6) and 8±1.2% and 6.9±0.4%, respectively, by day 3 (n=8).

Coomassie blue staining of polyacrylamide gels loaded with equivalent amounts of extracted myocyte proteins indicated no major changes in the protein pattern for up to 6 days in culture (Figure 3). However, a decrease in both the MHC and the actin content at day 5 was detected by densitometric analysis (MHC and actin values expressed as a percentage of total protein were, respectively, 19.6±0.4% and 13.9±0.3% at day 5 vs. 22.1±0.8% and 17.2±1.6% at day 0, p<0.05; number of different cultures=5).

\textbf{Effects of Adrenergic Stimulations on Protein Synthesis of Adult Cardiac Myocytes}

The effects of adrenergic stimulation on adult myocyte growth were analyzed by addition to the culture medium of either isoproterenol (\(\beta\)-adrenergic...
agonist) or norepinephrine (α-β-adrenergic agonist) at a final concentration of 1 nM–10 μM for 3 days.

The addition of adrenergic agonists to cultured adult myocytes, which are known to be quiescent,17 caused spontaneous contractions of some cells for a few minutes in contrast to those observed in neonatal myocytes.3 Adrenergic stimulation had no effect relative to the controls on the pattern of constitutive proteins, when analyzed by PAGE–Coomassie blue staining (not shown). The treatment with low concentrations (1–10 nM) of isoproterenol for 3 days altered neither the cell density nor the percentage of rod-shaped cardiac myocytes when compared with the untreated cells (Table 2). As with isoproterenol, treatment of myocytes with 1–10 nM norepinephrine had no effect on the cell viability (27±5 cells/mm2; 59±2% of rod-shaped myocytes). Both hormones at a concentration of 10 nM increased the [14C]phe incorporation into total protein by 20% (p<0.05). These increases were inhibited (>90%) by the β-adrenergic antagonist propranolol, but neither the α1-selective antagonist prazosin nor the α2-selective antagonist yohimbine inhibited the effects of 10 nM norepinephrine (Figure 4). The specific activity of actin and MHCs, evaluated as described above, was not altered by 10 nM isoproterenol treatment (Table 2). To assess the hormonal stimulation of noncontractile protein synthesis, the specific activity of proteins migrating between MHCs and actin on the PAGE was quantitated in two experiments. Values increased significantly in the presence of the hormone (+29% and +35% when compared with controls). No significant data could be obtained when each noncontractile protein band was separately analyzed, because of their relatively low abundance.


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A theoretical estimation of the percentage of total radioactivity incorporated into MHC and actin (specific activity multiplied by percent of protein divided by specific activity of total protein) in the presence or absence of isoproterenol yields differences too low to be detected on gels loaded with the same amount of radioactivity. Indeed, when the respective percentages of MHC and actin content on a 10% gel (see above) are considered, the [14C]phe incorporation into MHCs and actin will represent, respectively, 3.4% and 3.4%.

FIGURE 3. Gel electrophoresis showing patterns of stored proteins from adult cardiac myocytes as a function of time in culture. The same amount of total proteins was loaded onto each lane of 10% polyacrylamide gel and stained with Coomassie blue. Note the absence of major changes in the protein pattern during 6 days in culture. MHC, myosin heavy chain; Act, actin; Tm, tropomyosin.

TABLE 2. Effects of Isoproterenol on the Characteristics of 3-Day Cultured Adult Rat Cardiac Myocytes

<table>
<thead>
<tr>
<th>Cell characteristics</th>
<th>Control</th>
<th>Isoproterenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density (cells/mm²)</td>
<td>34.3±3.3 (17)</td>
<td>35.0±6.0 (11)</td>
</tr>
<tr>
<td>Rod-shaped myocytes (%)</td>
<td>58.8±1.5 (17)</td>
<td>61.7±2.0 (11)</td>
</tr>
<tr>
<td>Protein synthesis (dpm/µg protein)</td>
<td>219±6 (20)</td>
<td>266±13* (9)</td>
</tr>
<tr>
<td>Myosin heavy chain</td>
<td>38±5 (5)</td>
<td>39±7 (5)</td>
</tr>
<tr>
<td>Actin</td>
<td>44±1.4 (5)</td>
<td>51±9 (5)</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Myocytes were incubated in the presence or absence of 10−8 M isoproterenol for 3 days. [14C]Phenylalanine (450 mCi/mmol) was added to the medium (1 µCi/ml) during the last 24 hours in culture. Total protein synthesis was evaluated as in Table 1. The specific activity of myosin heavy chain and actin was determined by loading 10% polyacrylamide gels with total protein extracts. Bands of myosin heavy chain and actin were excised, the proteins were electroeluted, and trichloroacetic acid–precipitable fractions were used for protein determination and scintillation counting. The number of different cultures is indicated in parentheses.

*Significantly different from control data with p<0.05 (Scheffe’s test).

FIGURE 4. Bar graph showing effect of norepinephrine (NE), isoproterenol (Iso), and adrenergic antagonists on total protein synthesis in adult myocytes. Myocytes were cultured for 3 days in the presence of 10 nM Iso or 10 nM NE with or without 10 µM propranolol (Prop), 0.1 µM prazosin (Praz), or 0.1 µM yohimbine (Yoh). The incorporation of [14C]phe into total myocyte proteins was determined as in Table 2. *p<0.05; **p<0.01.
stimulation of myofibrillogenesis, on adult rat cardiac myocytes in culture.

Adult rat cardiac myocytes cultured for 1 week by Eckel’s technique maintained their in vivo morphology and did not go through the dedifferentiation process defined by others (for a review, see Reference 17). As a matter of fact, a slight decrease in the relative amount of contractile proteins was observed only by day 5 of culture; in other models, it occurs as soon as day 2. This could be secondary to differences in the culture medium or to species specificity of each model. Methods used for the evaluation of the protein synthetic activity in these cultured adult myocytes ranged from the simple qualitative analysis of [35S]methionine incorporation into electrophoretically resolved proteins to a more detailed analysis of quantitative changes in the [14C]phe incorporation into both total protein and electrophoretically purified proteins.

Total protein synthesis rapidly decreased during the first days of culture and reached a steady-state level after 3 days. It emerges from data presented here (Figure 2 and Table 1) that the decrease in synthetic activity of total proteins (30%) mainly reflects the decline in synthesis of the myofibrillar proteins (>50%). The half-life of both MHCs and actin is longer than 5 days in adult rat ventricle (for a review, see Reference 24), and the fact that the relative content of MHCs and actin remained constant while their specific activity decreased suggests that the degradation processes are not prominent in our experimental conditions.

A 3-day adrenergic stimulation induces a clear trophic effect in adult (Table 2 and Figure 4) as in neonatal rat myocytes. This contrasts with studies on isolated adult cat myocytes, in which no specific adrenergic agonist effect was observed. This discrepancy is probably species dependent but may result from a difference in the experimental conditions. In the study by Cooper et al., cat myocyte adrenergic treatment was started 3 days after plating, and the hormonal effects were analyzed after 11 days of stimulation; in our study, the agonist treatment began at the onset of myocyte culturing and lasted only 3 days. With longer time in culture, adult myocytes could have changed some of their characteristics even if they did not dedifferentiate; these changes could account for differences between these two studies.

The increase in total protein synthesis (+20%) observed in adult cells after 3 days of adrenergic stimulation was smaller than that observed in neonatal cells (>50%). However, it should be pointed out that the β-adrenergic stimulation of adult myocytes had no effect on the synthesis of contractile proteins (Figure 5 and Table 2), which represents around 50% of the total protein. Therefore, the 20% increase in total protein synthesis reflects, with an underestimation, the increase in synthesis of nonmyofibrillar proteins (around 30%). Thus, in adult myocytes, the synthesis of nonmyofibrillar proteins may be trig-

**Figure 5.** Gel electrophoresis showing pattern of proteins labeled with [35S]methionine from cardiac myocytes treated with β-adrenergic agonists. The same amount of [35S]radioactivity (3×10^6 dpm) was loaded on each lane of the 10% polyacrylamide gel. No major differences were observed among cells treated with 10 nM isoproterenol (Iso), 10 nM Iso plus 10 μM propranolol (Prop), and the controls. Note that 10 μM Iso inhibited myosin heavy chain (MHC) synthesis. Arrows point to MHC and actin (Act).

and 2.8% in control versus 2.9% and 2.6% in isoproterenol-stimulated cells. However, to determine if the increased specific activity of noncontractile proteins was related to a preferentially stimulated synthesis of some unique proteins, [35S]methionine-labeled proteins extracted from myocytes after a 3-day β-adrenergic stimulation were analyzed on 10–20% gradient gels loaded with the same amount of radioactivity. The electrophoretic patterns did not significantly differ from those of controls (Figure 5); this finding indicates that isoproterenol harmoniously stimulates the synthesis of most noncontractile proteins.

Higher concentrations of norepinephrine or isoproterenol (1 μM) had a toxic effect on adult cardiac myocytes as indicated by the decrease in the myocyte density after 3 days in culture (23.6±4 cells/mm² vs. 34.3±3.5 in control, p<0.05), the absence of an effect on [14C]phe incorporation (218±9 cpm/μg protein vs. controls in Table 2), and the relative decrease in the [35S]methionine labeling of MHCs (Figure 5).

**Discussion**

In this study, we have shown that low doses of adrenergic agonists acting through the β-receptors have a trophic but not hypertrophic effect, that is,
gered by adrenergic stimulation; in neonatal cells, adrenergic agonists also stimulate the synthesis of contractile proteins.3,4,25 The differences between the increased levels of adrenergic-dependent protein synthesis in the two types of cells most probably arise from the specific pathways mediating growth in neonatal and adult cardiocytes. Indeed, it is clear from our results that the adrenergic-induced growth of adult cardiac myocytes is only mediated through β-adrenergic receptors and not through the α1-adrenergic receptors as in neonatal cells.3,4 Thus, adrenergic stimulation of adult myocyte growth would depend on a transduction event via a cyclic AMP pathway19 and in neonatal myocytes probably by the phosphatidylinositol turnover.26 The reexpression of the “fetal program” involving the proto-oncogene myc and the α-skeletal actin gene occurs when the development of cardiac myocyte hypertrophy is induced either by an α1-adrenergic stimulation in neonatal cells25,27 or by a pressure overload in adult myocardium.28–30 The study of the qualitative changes in isolated adult rat myocyte gene expression in response to a β-adrenergic stimulation would indicate whether these diverse effects are mediated through common or distinct intracellular pathways in neonatal and adult myocytes.

In isolated adult rat myocytes maintained in culture, low doses (10 nM) of isoproterenol or norepinephrine induce trophic effects; high concentrations (1–10 μM) of the agonists have toxic effects (Figure 5). In a similar way, long-term treatment with low doses of isoproterenol stimulates in vivo cardiac hypertrophy without evidence of necrosis10; high concentrations of this agonist induce massive tissue lesions.31 Therefore, these data suggest that low doses of the agonists would be effective in inducing long-term responses such as the development of growth and that high doses of β-adrenergic agonists would be mainly effective in inducing a short-term myocyte response, such as positive inotropic and chronotropic effects. Therefore, in adult rats, the increase in total protein synthesis appears to be directly mediated through β-adrenergic stimulation and not through its inotropic or chronotropic effects since it occurs even when cardiac myocytes are not beating, as shown in this study.

In adult cardiac quiescent myocytes, the synthesis of contractile proteins rapidly decreased with time, and β-adrenergic stimulation by itself was unable to reverse the process. This supports the hypothesis that in vitro as well as in vivo contractile activity of cells is a major factor controlling the synthetic activity of contractile proteins in adult myocytes, as shown in this study and others.5,6,32 and in neonatal myocytes.16,33–36 In neonatal rat cardiac myocytes, a single factor such as an α1-adrenergic agonist4,35 or thyroid hormone36 is able to induce cell hypertrophy whatever the cell contractile activity. In contrast, adult cells require hormonal factors acting together with contractile-dependent regulatory mechanisms to induce myocyte hypertrophy.10,37–39 Indeed, a β-adrenergic stimulation increases the myofibrillar protein synthesis in contracting10,37 but not in quiescent (our present study) adult myocytes. Further studies are required to determine the mechanisms that control myofibrillar protein synthesis in neonatal and adult cardiac myocytes.

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